ACTA ZOOLOGICA

INTERNATIONELL TIDSKRIFT FÖR ZOOLOGI

UTGIVEN AV

NILS HOLMGREN OCH JOHN RUNNSTRÖM

TJUGUFJÄRDE ÅRGÅNGEN

VOL. 24 - 1943

VOL.
24
1943

GRUNDAD ÅR 1920 AV NILS HOLMGREN UNDER MEDVERKAN AV

O. ABEL, Wien.
A. APPELLÖF, Uppsala.
W. BATESON, London.
F. A. BATHER, London.
I. BOLIVAR, Madrid.
K. BONNEVIE, Kristiania.
E. W. MC BRIDE, London.
A. BRINKMAN, Bergen.
O. CARLGREN, Lund.
C. E. MC CLUNG, Philadelphia.
E. G. CONKLIN, Princeton.
A. DENDY, London.
F. DOFLEIN, Breslau.
L. DOLLO, Bruxelles.
H. FEDERLEY, Helsingfors.
E. GIGLIO-TOS, Cagliari.
C. GOLGI, Pavia.
S. GOTO, Tokyo.
B. GRASSI, Roma.
R. G. HARRISON, New Haven.
E. HEROUARD, Paris.
R. HERTWIG, München.
K. HESCHELER, Zürich.
J. HJORT, Kristiania.
C. J. van der HORST, Amsterdam.
F. A. JANSSENS, Louvain.
J. B. JOHNSTON, Minneapolis.
C. U. ARIËNS KAPPERS, Amsterdam.
R. KOEHLER, Lyon.
C. A. KOFOID, Berkeley.
A. KROGH, Köpenhamn.
W. LECHE, Stockholm.
F. R. LILLIE, Chicago.
J. LOEB, New York.
A. LUTHER, Helsingfors.
E. L. MARK, Cambridge, U. S. A.
TH. MORTENSEN, Köpenhamn.
TH. ODHNER, Stockholm.
H. F. OSBORN, New York.
H. PRZIBRAM, Wien.
P. RAMON Y CAJAL, Zaragoza.
S. RAMON Y CAJAL, Madrid.
E. REUTER, Helsingfors.
W. ROUX, Halle.
F. SARASIN, Basel.
C. PH. SLUITER, Amsterdam.
A. SMITH-WOODWARD, London.
H. G. STEHLIN, Basel.
TH. STUDER, Bern.
H. WALLENGREN, Lund.
M. WEBER, Amsterdam.
C. J. WESENBERG-LUND, Hillerød.
W. M. WHEELER, Boston.
E. B. WILSON, New York.
A. WIRÉN, Uppsala.
F. ZSCHOKKE, Basel.

STOCKHOLM · ALBERT BONNIERS FÖRLAG

VOL
24
194

STOCKHOLM. ALB. BONNIERS BOKTRYCKERI 1944

VOL.
24
1943

INNEHÅLL

HOLMGREN, NILS: Studies on the Head of Fishes. Part IV 1

ALEXANDROV, W. J. und NASSONOV, D. N.: Über die Ursachen der kolloidalen Veränderungen des Protoplasmas 189

VOL
24
194

VOL.
24
1943

STUDIES ON THE HEAD OF FISHES

AN EMBRYOLOGICAL, MORPHOLOGICAL, AND PHYLOGENETICAL STUDY

BY

NILS HOLMGREN

PART IV.

GENERAL MORPHOLOGY OF THE HEAD IN FISH

CONTENTS.

Introduction 3
Material and method 3
On the nature of the trabecula cranii 5
The origin of the trabecula and of the polar cartilage in *Squalus acanthias* . 5
The origin of the trabecula and of the polar cartilage in *Salmo salar* . . . 7
The origin of the trabecula and of the polar cartilage in *Acipenser* 15
The origin of the trabecula and of the polar cartilage in *Amia* 21
The relations of the carotid, the efferent pseudobranchial and the ophthalmic arteries to the polar cartilage and the trabecula in Selachians and Teleostomians 22
The relations of the trabecular bar and the palato-quadrate in sharks 28
The relations of the trabecular bar and the palato-quadrate and certain dermal bones in *Acipenser* 29
The relation of the palato-quadrate to the tentacles in *Acipenser* 34
The relation of the palato-quadrate and the trabecula and certain dermal bones in *Amia* 35
The relations of the trabecula and the palato-quadrate in teleosts 40
Comparison and conclusion 43
The mandibular and hyoid arches 45
Observations on the branchial arches in Teleostomians and Selachians . . . 45
The morphology of the mandibular arch in sharks and rays 51
The »postpalatinum mediale» *(Bugajew,* 1930) 66
The morphology of the hyomandibula in sharks and rays and its relation to the auditory capsule and to the mandibular arch 67

The hyomandibula in Teleostomians 76
a) Some introductory remarks about the morphology of the hyomandibula . 79
b) The location of the early rudiment of the hyomandibula and the relation to the principal head vein 80
In sharks and rays 80
In *Acipenser* 80
In *Amia* 81
c) The early relations of the rudiment of the hyomandibula to the hyomandibular nerve 82
In Selachians 82
In *Acipenser* 82
In *Amia* 82
In *Lepidosteus* 82
In *Salmo* 83
d) The development of the hyomandibula in Teleostomians 83
In *Acipencer* 83
In *Amia* 86
In *Lepidosteus* 87
In *Polypterus* 88
In *Salmo* 88
e) The »hyomandibular muscle» 94
f) The hyomandibula in *Polypterus* and Palaeoniscids 94
g) The hyomandibula of the Crossopterygians 100
h) Summary concerning the hyomandibula 100
The theory of *Schmalhausen* and others concerning the different position of the hyomandibula in Selachians and Teleostomians 102
The stylohyal problem 104
The symplecticum 107
The relations of the mandibular arch to the hyomandibula and to the neurocranium, phylogenetically considered 108
The head veins 117
The head veins in *Squalus acanthias* embryos 119
The head veins in *Acipenser* embryos 127
The head veins in *Amia* embryos 134
The head veins in *Lepidosteus platystomus* embryos 136
The head veins in *Salmo salar* embryos 138
Summary account of the head veins 139
The auditory capsule and the glossopharyngeal nerve in Selachians 140
The posterior myodome 148
1) The lateral commissure 149
2) The parachordal question in *Holostei* and Teleosts 150
3) The relation of the lateral wall of the brain-case and the trabecular system in *Amia, Lepidosteus* and *Salmo* 152
4) Theories on the morphological nature of the posterior myodome . . . 154
5) The ontogenetical development of the interorbital septum and the myodome in *Salmo salar* 156
Stage, 41 days old 156
Stage, 44 days old 157
Stage, 52 days old 158
Stage, 58 days old 159
Stage, 23 mm body length 161
Stage, 30 mm body length 162
6) The septum interorbitale and the myodome in *Amia* and *Lepidosteus* . . 162
7) Teleosts without a functional myodome 164
8) Fishes with only the dorsal compartment of the myodome 165
9) Fishes with a complete teleostean myodome 166
10) Conclusions 166
Concernering the anterior boundary of the orbit in Selachians and Teleostomians . . 170
The orbital capsule and its relations to the skull 171
1) In *Petromyzon* and in Selachians 171
2) In Teleostomians 177
Ligamentum tenaculum oculi 180

VOL. 24 1943

INTRODUCTION.

The present paper is the fourth part of a series, "Studies on the Head of Fishes", of which the first part treated the ontogenetical development of the skull in selachians, the second the comparative anatomy of the skull of selachians and the third the phylogeny of the elasmobranch fishes. During the progress of these investigations I came across a great number of problems of general morphological importance. These mostly were problems already many times discussed by different workers, but which nevertheless invited to further discussion, but there also appeared a number of new problems and interpretations, inviting to further research. In the first part of this series I have set forth a considerable number of theories and explanations, applicable on the conditions in selachians, which called for further investigations in order to ascertain if they are of general importance and acceptable as general theories. For this purpose I have widened my embryological researches to comprise a number of ganoids and teleosts. This fourth and last part of my "Studies on the Head of Fishes" thus will contain a considerable amount of original embryological researches especially on *Acipenser, Amia* and *Salmo,* but also on *Lepidosteus* and a considerable number of teleosts. For comparison an amount of palaeontological material, gathered from the literature, has been used.

My work has been facilitated through stipends from "Stiftelsen Lars Hiertas minne", "Gustaf och Anna Retzius fond för vetenskaplig forskning", "Venner-Grenska Samfundet", "Längmanska kulturfonden" och "Bendixsonska fonden", To the boards of all these foundations I here wish to express my most respectful and warmest gratitude. I wish also to tender my hearty and sincere thanks to my friends Professor E. Stensiö and lic. phil. G. Svensson for the very valuable opportunities they have given me to discuss with them many a question of general or special morphological interest. I am also very much indepted to my friend Dr. O. Nybelin of Gothenburg for forwarding to me two unique embryos of *Myxine.* To my assistants lic. phil. Gustav Svensson and cand. phil. Bertil Lekander I also wish to tender my sincere thanks for all the conscientious work they have done on my behalf.

MATERIAL AND METHOD.

In addition to the material (of Selachians), of which account was given in the first part of this work (Holmgren, 1940) I have investigated embryos of teleostomian fishes according to the following list.

Acipenser Gyldenstaedti: Stages 7.8, 9, 10, 13.5, 14, 19 mm body length.

Acipenser ruthenus: 7, 7.2, 7.5, 8, 8.5, 8.6, 8.8, 9, 12, 12.5, 13, 14, 15, 15.5, 16, 26, 32, 36, 44 mm and 7, 23, 27, 29, 39, 41, 47, 50, 52, 60, 70 days old and 21 egg-stages (mixed stages).

Acipenser stellatus: 10, 13, 14.5, 17, 20, 21 mm stages.

Amia calva: 6, 6.5, 7, 7.1, 7.4, 7.5, 8, 8.2, 9, 9.2, 9.5, 9.9, 10, 10.4, 11, 11.5, 12, 12.5, 13, 13.2, 13.8, 15.7, 16.1, 16.5, 17, 18.2, 20, 22, 56 mm stages and a great number of series of different stages, but without specification.

Lepidosteus platystomus: 6, 6.2, 6.8, 8, 8.3, 9, 9.3, 10.1, 11, 12, 12.3, 16, 16.5, 17.5, 18, 18.3, 18.5, 18.7, 19, 19.2, 19.3, 20.4, 21.5, 25, 33.4, 35.2, 40.7, 44.2, 54.2, 60, 63.1, 65.4, 70 mm stages.

Polypterus bichir: 45, 125 mm.

Polypterus lapradei: 80 mm.

Polypterus senegalus: 37, 45, 101 mm.

Salmo salar: 235 series of sections from gastrulation to 30 mm body length. 5 different sets. Reared in running tapwater at 10—12° C. or at 4° C. Fixation three times a day.

In addition to the enumerated material I have used series of the following fishes:

Amiurus nebulosus (1 ser.), *Ammodytes tobianus* (2 ser.), *Arius commersoni* (1 ser.), *Anguilla vulgaris* (2 ser.), *Belone acus* (2 ser.), *Bothus maximus* (6 ser.), *Calamoichthys calabaricus* (1 ser., adult), *Ceratodus forsteri* (39 ser.), *Clupea harengus* (13 ser.), *Clupea sprattus* (2 ser.), *Cobitis taenia* (2 ser., adult), *Coregonus lavaretus* (4 ser.), *Corydoras paleatus* (44 ser.), *Cottus bubalis* (1 ser.), *Cyclopterus lumpus* (25 ser.), *Esox lucius* (69 ser.), *Gadus merlangus* (2 ser.), *Gadus morrhua* (1 ser.), *Galidichthys* sp. (11 ser.), *Gobius* sp. (3 ser.), *Gymnarchus niloticus* (8 ser.), *Heterotis niloticus* (2 ser.), *Leuciscus rutilus* (12 ser.), *Marcusenia rudebeckii* (1 ser.), *Nerophis* sp. (3 ser.), *Petrocephalus brevipedunculatus* (2 ser.), *Protopterus annectens* (a great number of stages), *Salmo salvellinus* (100 ser.), *Scomber scomber* (1 ser.), *Siphostomo typhle* (2 ser.), *Spinachia spinachia* (2 ser.), *Sygnathus acus* (10 ser.), *Trachurus trachurus* (2 ser.) and *Zoarces viviparus* (16 ser.)

My work is chiefly based upon serial sections stained according to the Azan-Mallory method. Graphical reconstructions, based upon these series, have been used to a large extent. Since at least twentyfive years ago I have used photographical pictures (paper negatives) of the sections as basis for the reconstructions instead of pencil drawings.

STUDIES ON THE HEAD OF FISHES

ON THE NATURE OF THE TRABECULA CRANII.

A question of primary importance to the very theory of the morphology of the skull is that of the origin of the polar cartilages and trabeculae cranii. Upon the answer to this question depends the conception of neurocranial as well as viscerocranial structures. I shall therefore take up this question here.

The statement of KOLTZOFF (1902) that the trabeculae of *Petromyzon* are derived from the three foremost cephalic somites led SEWERTZOFF (1916) to deny the homology of the trabeculae of *Petromyzon* with those of Gnathostomians, where they are said to be mesenchymatic structures not derived from somites. This contention gave rise to a search of the homologues of the gnathostomian trabecles in *Petromyzon*. This search resulted in the assumption that the dorsal prenasal muco-cartilage plate of the *Ammocoetes* larva were the representatives of the latter. I have given some preliminary reasons against this theory (HOLMGREN—STENSIÖ, 1936) without considering it in detail. In the present paper I shall consider the question from partly new points of view, investigating the origin of the trabecula and polar cartilage in Gnathostomians.

VOL. 24 1943

The starting point of SEWERTZOFF's contention was KOLTZOFF's statement, that the trabecles of *Petromyzon* were somitic of origin. Meanwhile, the belgian embryologist H. DAMAS has recently (1943) published an excellent paper on the development of *Petromyzon*, in which strong reasons are given for the interpretation of the trabecles of *Petromyzon* as derived from the ectomesoderme (neural crest).

The origin of the trabecula and polar cartilage in Squalus acanthias.

The problem of the origin of the trabeculae and polar cartilages of the Selachians has not yet been satisfactory solved. As far as I can find, only HALLER (1924), MATVEIEFF (1925) and DE BEER (1931 b) have attempted to trace their origin. In the first part of this work (HOLMGREN, 1940) I too have made a contribution to this question, which may perhaps throw some fresh light upon the problem.

According to HALLER, the posterior part of the trabeculae (polar cartilages) is formed in the "Hypophysenpolster", the origin of which was not determined. The anterior part of the trabeculae (trabeculae sensu strictiori) was derived from the maxillary process of the mandibular arch. DE BEER's contention was similar. As to the polar cartilage, however, he was not able to satisfy himself from the material at disposal that the polar cartilage rudiment arises from the mandibular arch, "probable though it may be". According to DE BEER, the

trabecles are derived from the mesenchyme of the maxillary process and are thus "visceral" structures. MATVEIEFF's ideas of the polar cartilage and trabecula are different. According to MATVEIEFF, the hyoid somite forms partly the foremost portion of the parachordal plate, partly the polar cartilage. The mandibular segment and the premandibular furnish the material for the trabecle of which the anterior end is formed by mesenchyme not somitic in origin. Thus MATVEIEFF considers the polar cartilages and the principal part of the trabeculae to be derived from the anterior head somites, an idea completely opposed to that of HALLER and DE BEER.

According to my experiences, the embryonic stages used by the named authors in their investigations have been throughout too far advanced.

In the first part of this paper (HOLMGREN, 1940) I have described the earliest stages of the development of the trabecula. At a 5.7 mm stage of *Squalus acanthias,* in which the mesenchyme of the mandibular arch is easily distinguishable from that derived from the somites, the future trabecle is found behind the vesicle of PLATT, from which it is differentiated as a strand of cells, as yet loosely connected with the vesicle. From the 12 mm stage this strand is discernable as the developing rudiment of the trabecula. This rudiment is thus entomesodermal, being derived from the vesicle of PLATT[1], which is considered to belong to the premandibular somite. But there are also indications that ectomesodermal cells may have entered the rudiment as it has no sharp outlines. It seems, however, clear from my observations that the rudiment of the trabecula cranii contains at least a core of somitic mesenchyme and that it originates quite independently of the maxillary process. Later (already in the 12 mm stage) it gains contact with the mandibular arch and seems to fuse on to it, at least frontally where a trabecular process (the future orbital process) forms the connecting link between the two structures (HOLMGREN, 1940).

The development of the polar cartilages has also been followed in the same paper (HOLMGREN, 1940). There it has been demonstrated beyond any doubt that the polar cartilage is derived from the mandibular somite (see for instance figures 34 and 38 in that paper).

Thus the early rudiments of the trabecula and the polar cartilage are both of somitic origin, products of the premandibular and mandibular somites respectively, and the connection of the trabecula with the mesenchyma of the mandibular arch (palato-quadrate) due to secondary fusion. Meanwhile the trabecula grows by means of the apposition of ectomesodermal cells. This is especially the case in regards to its frontal growth, where the so-called rostral plate seems to be exclusively of ectomesodermal origin.

[1] The premandibular mass of DOHRN also seems to have something to do with the development of the trabecular rudiment.

The origin of the trabecula and of the polar cartilage in Salmo salar.

In *Salmo fario,* investigated by DE BEER (1931 b), that author maintains that the trabecula originates as a proliferation from the inner side of a "mesenchymatous plaque", which is continuous with the mesenchyme of the mandibular arch caudally. Thus the trabecula should be a visceral structure. DE BEER, however, discusses at some length the possibility of a somitic origin of the trabecula: "Posteriorly the mesenchyme of the trabecular rudiment becomes less dense, and it comes into contact with the strand of mesenchyme which... represents the transverse commissure between the two premandibular head cavities.[1] It might be supposed that this contact was evidence for the origin of the trabeculae from the transverse commissure and so from the premandibular somite. But this connection of the trabecular rudiment with the mesenchymatous plaque is so dense and intimate, while that with the transverse commissure is so ephemeral, that there can be little doubt that it is from the former and not from the latter that the trabeculae are formed." My experiences with regard to the origin of the trabecula in *Salmo salar* widely disagree with the results of DE BEER and give a quite different idea of the nature of the trabecula. As this question is of primary importance for the morphology of the skull I propose here to take up the question at some length. For the investigation I had at my disposal 235 series of sections of embryos from a very rich material representing five different sets of eggs fertilized in the laboratory and reared at different temperatures. Fixations were made three times a day. The most favourable set was reared at 4° C. At this temperature the development goes very slowly (as in nature)[2], and therefore the developmental series could be without any perceptible gap. In this set no variations were noted, whereas variations were abundant in sets reared in running tapwater. (Compare DE BEER, 1927).

In order to advance the question as to the origin of the trabecular system it is necessary to begin the investigation with such young developmental stages that the mesoderm of the head region is still not quite differentiated, and to follow its transformations until the skeletal pieces and muscles of the head are more or less formed. It is also necessary to follow simultanously the development of the neural crest material.

The early development of the mesoderm of teleostean fishes is well known, especially from the works of GORONOWITSCH (1885), HENNEGUY (1888), BOYER (1892), JABLONOWSKI (1898), SWAEN and BRACHET (1899), BOEKE (1902) and others,[3] so that it is not necessary to describe it in detail. Some

[1] In *Salmo salar* and *salvellinus* no head cavities occur (HOLMGREN's remark).
[2] The development (up to the time of hatching) in running tapwater (10—12° C.) has taken 60 to 50 days, in 4° water 120 days and more.
[3] It is not necessary to consider the modern ideas of the earliest development of the mesoderme in the teleosts (PASTEL).

points, however, may be emphasized. The frontal prechordal part of the entoderm at a certain young stage of *Salmo salar* is a layer of cells, thin in the medial line and thicker laterally. In the most frontal part of this layer a great mass forms ["vordere Entodermmasse" (JABLONOWSKI)] in front of the brain rudiment. This mass decreases during the following development owing to the transformation of its cells into mesenchyma (ento-mesoderm) (JABLONOWSKI). This frontal entoderm corresponds to the "präorales

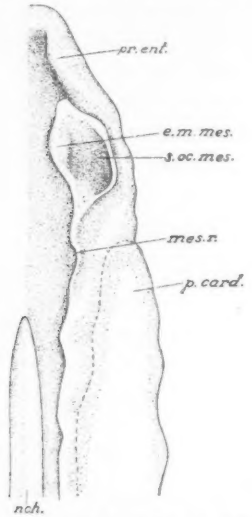


Fig. 1. *Salmo salar*, embryo 8 days 19 hours old, 10—12° C. Reconstruction of the mesoderme of the head region, dorsal aspect.

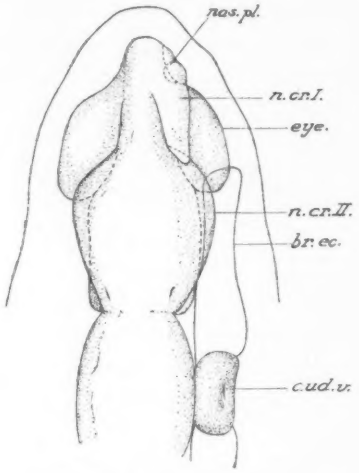


Fig. 2. *Salmo salar*, embryo 8 days 19 hours old, 10—12° C. Reconstruction of the neural crests of the head region, dorsal aspect.

Entoderm" of KUPPFER (1893) and perhaps also to the "premandibular mass" of DOHRN in Selachians.

The mesoderm is separated from the primary entoderm from behind, (chordo-meso-entoderm) about to the level of the anterior border of the eye-vesicle. Medially to the eye the mesoderm plate is considerably thickened or ridge-like (fig. 1). From this ridge a lamella runs laterad embracing the eye-vesicle ventrally and laterally (and caudally), forming a bowl in which the vesicle rests. Behind the eye the mesoderm plate has a high ridge which projects dorsad on the side of the brain. Laterally the plate thins out, extending to the border of the embryo. Somewhat more caudally the thin lateral part of the mesoderme is continued laterad as the rudiment of the pericardium.

There is no trace of a segmentation of the mesoderm plate of the head, but that part of the plate which is connected with the rudiment of the pericardium may perhaps represent a non-delimited mandibular somite (conclusion from the conditions in sharks).

For the purpose of the following description I will propose to introduce the terms *subocular mesoderme* (fig. 1 s.oc.mes.) for the subocular lamella and *eye-muscle mesoderme* (e.m.mes.) for the ridge inside the eye. (Both parts have been illustrated, for instance, by GORONOWITSCH (1885).

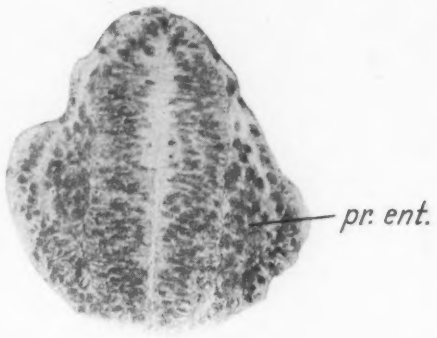


Fig. 3. *Salmo salar*, embryo 13 days 23 hours old, 4° C. Transversal section through the formost part of the head with the prefrontal entoderme.

At the stage (fig. 2) of mesodermal development represented in fig. 1, the neural crest is developing in the region of the future midbrain and "tweenbrain". The frontal portion (fig. 2 n.cr.I.) ventrally abuts on to the dorsal part of the eye-vesicle. More caudally the second crest (n.cr.II.) has grown down on the outside of the upper border of the mesodermal ridge (fig. 1 mes.r.) mentioned in preceding lines. In front of the eye-vesicle a not very clearly delimited nasal placode (nas.pl.) is present as an antero-lateral portion of a long placode-like ectodermal thickening above the eye. The future location of the gill-slits is marked by a long ectodermal thickening (br.ec.) extending from the eye to the auditory placode. In the entoderm a corresponding thickening signifies the location of the future branchial pockets.

In a following stage the mesodermal plate is much as before, but the eye-muscle mesoderm is broader. The frontal entoderm becomes transformed into

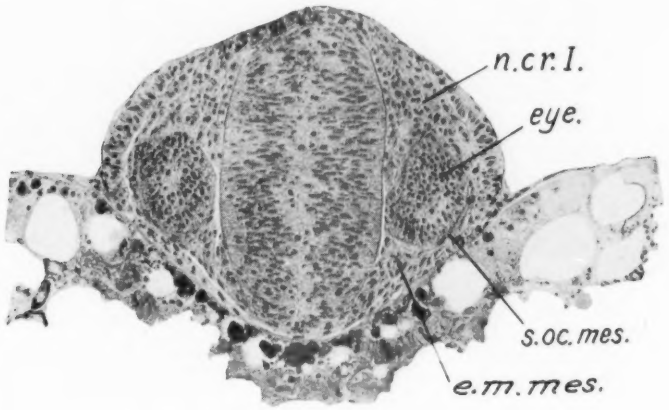


Fig. 4. *Salmo salar*, 29 days 9 hours, 4° C. Transversal section through the anterior neural crest and the premandibular somite.

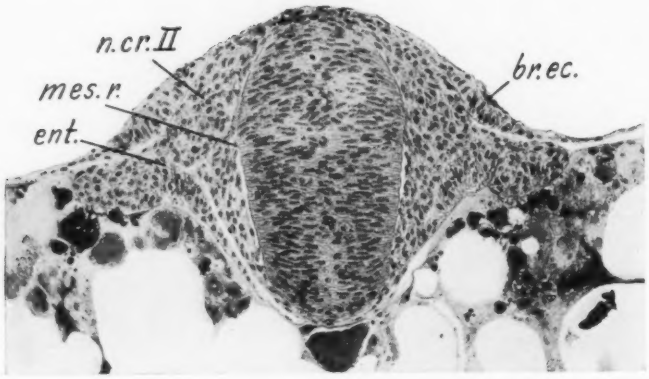


Fig. 5. *Salmo salar*, 29 days 9 hours, 4° C. Transversal section through the second neural crest.

a frontal mesenchyme lying in front of the eye (fig. 3).[1] The neural crest has now developed a frontal portion dorsally to the eye-stalk. The anterior part of this portion abuts on to the eye-vesicle. The posterior portion divides into two parts (n.cr.I.), the one descending inside the eye, the other outside it (fig. 4). The inside portion extends further ventrad than the outside one. Behind the eye the crest reaches so far ventrally as to come into contact with the entoderm (fig. 5).

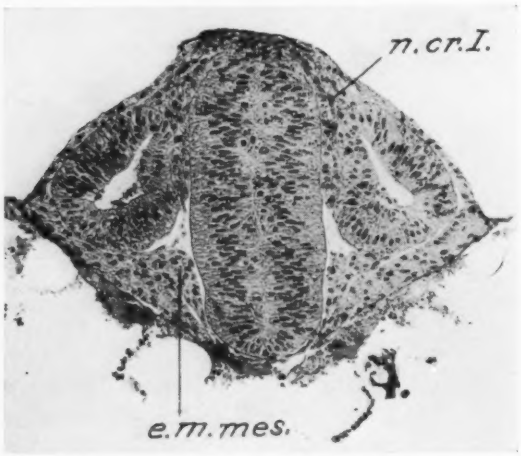


Fig. 6. *Salmo salar*, 9 days 13 hours, 10—12° C. Section through the anterior neural crest and the premandibular mesoderme. More advanced stage than that in fig. 4.

In the next stage the eye-muscle mesoderm has increased considerably. The inside portion of the neural crest has grown down between the eye and the brain more than in the preceding stage (fig. 6), but has not yet reached the eye-muscle mesoderm (e.m.mes.) (Compare BOEKE, 1902). In a somewhat more advanced stage it has reached this mesoderm (fig. 7), sometimes as a flattened hollow sac (n.cr.I.), sometimes as a solid lamella. Somewhat later (fig. 8) the medial lamella of the neural crest disintergrates,

[1] The frontal entoderm has the same position in relation to the eye as the vesicle of *Chiarrugi* in the shark embryo. As both structures belong to the preoral part of the entoderme, it is most probable that they are homologous.

its cells being dispersed (n.cr.I.), some cells connecting with the lateral sides of the brain, forming an extremely thin envelope for the brain, others connecting with the eye-vesicle and entering between this and the subocular mesoderm (n.cr.I.). The connection with the neural crest portion of the brain is almost lost. At this stage the eye-muscle mesoderm has grown to a considerable cell-mass (fig. 8), laterally merging directly into the subocular mesoderm.

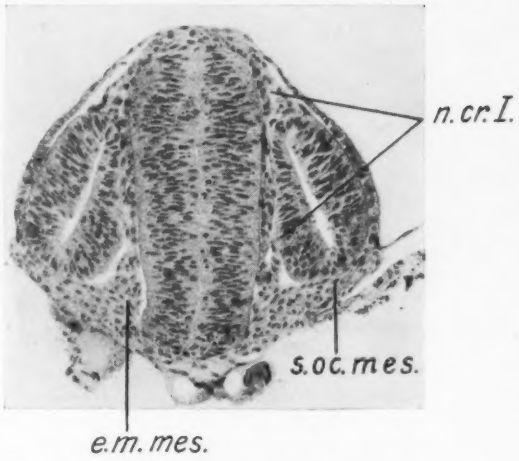


Fig. 7. *Salmo salar*, 25 days 21 hours, 4° C. More advanced stage than that of fig. 4. Transversal section through the anterior neural crest.

The medial ectomesodermal lamella between the brain and the eye, already dissolving in the preceding stage, is found a little later (fig. 9) building up blood-vessels. An upper and a lower orbital sinus are formed and vesselforming mesenchyma is entering the eye-muscle mesoderm splitting up this tissue from laterally into a dorsal part, the secondary eye-muscle mesoderm (fig. 9) and a ventral lamella continuous with the subocular mesoderm (s.oc.mes.). Frontally the subocular mesoderm continues without any line of demarcation into the "frontal mesoderm", viz. the mesoderm or mesenchyme resulting from the disintegration of the "vordere Entodermmasse". This frontal mesoderm extends from the nasal placode frontally covering the ventral part of the sides of the head in front of the eye, narrowing below the eye-stalk, where it approximately joins the subocular mesoderm. Fig. 10 is a reconstruction of the mesodermal (ento-mesodermal) structures of the head at this stage (17 days 22 hours old at about 8° C.). In the reconstruction there is no boundary between the fron-

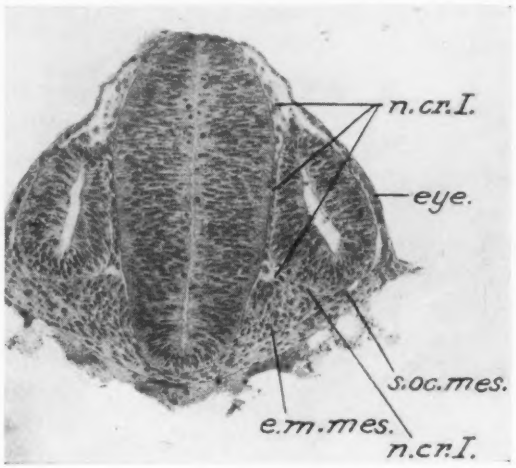


Fig. 8. *Salmo salar*, 25 days 21 hours, 4° C., more advanced stage than in fig. 7. Transversal section through the neural crest and the premandibular mesoderme.

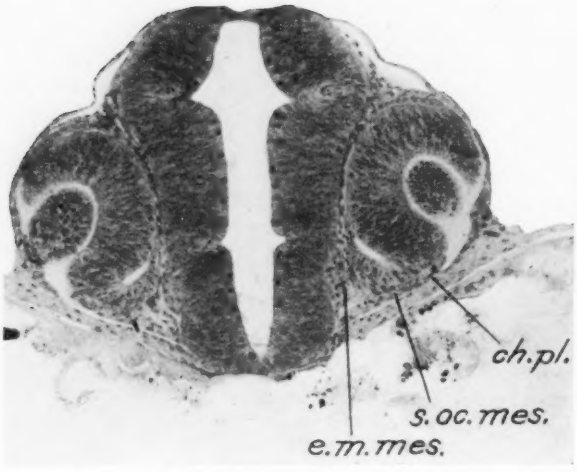


Fig. 9. *Salmo salar*, 32 days 9 hours, 4° C. Transversal section level with the eye.

tal mesoderm (fr.mes.) and the subocular (s.oc.mes.). The subocular mesoderm sends off a lamella through the ocular fissure to the interior of the eye-bulb, where it forms the rudiment of the plexus chorioideus (figs. 9, 10 ch.pl.) or campanula halleri. With this rudiment follow fine blood-vessels, probably ectomesodermal in origin. The postero-mesial corner of the subocular mesoderm is somewhat thicker than the rest and represents the initial rudiment of the trabecula cranii (figs. 10, 11 a, trab), as will be evidenced by the following stages. The eye-muscle mesoderm (e.m.mes.) forms a bulky square-cut mass of densely crowded cells inside the posterior part of the eye (figs. 10, 11 a). A strong commissure (the premandibular commissure) (fig. 10 com.) joins the contralateral masses behind the future infundibulum of the brain. The lateral parts of the postocular mesoderm forms an indistinctly delimited mesoderm mass representing a mandibular "somite" (figs. 10, 11 b, som.II.) which sends a process frontad above the posterior part of the eye. This process (fig. 10 m.o.s.) is the rudiment of the obliquus superior muscle of the eye. From the "somite" a muscular process (m.pr.md.), formed by densely crowded cells, enters the oral arch as a well delimited central core in this arch. Behind the mandibular somite follows a cylindrical mesodermal mass frontally well and caudally indistinctly delimited and representing a hyoid "somite" (som.III.). The frontal part is the

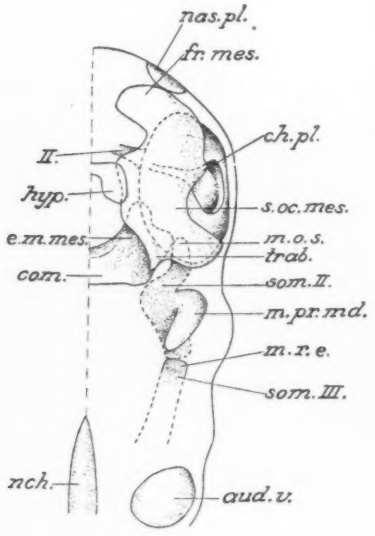


Fig. 10. *Salmo salar*, 17 days 22 hours, 10—12° C. Reconstruction of the mesoderme, ventral aspect.

rudiment of the external rectus muscle of the eye (m.r.e.).

In a following stage (48 days at 4° C.) (figs. 12, 13) the frontal mesoderm (fr.mes.) has joined its counterpart by means of a thin subepidermal cell-layer below the brain. The subocular mesoderm (s.oc.mes.) has a chorioidal portion (ch.pl.) as before. The postero-mesial border of the plate has differentiated a thicker border, which is the rudiment of the trabecula (trab.). This rudiment is broader frontally than caudally. Outside the trabecular rudiment there is a thickened and cellularly differentiated portion of the mesodermal plate, which represents the, at first visible, rudiment of the inferior obliquus muscle of the eye. The eye-muscle mesoderm (e.m.mes.) has grown considerably but has not yet differentiated any muscles. The mandibular "somite" is as before, but its muscular rudiments show a more advanced development. The mandibular somite (som.II.) is seen from the lateral side in fig. 13, where it forms an indistinctly delimited cylindrical cell-mass behind the eye. The hyoid "somite" is very indistinct, except frontally, where the rudiment of the rectus externus muscle (m.r.e.) is better outlined.

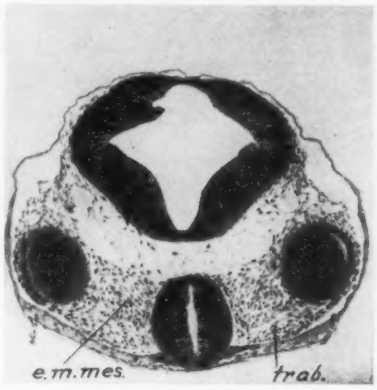


Fig. 11 a. *Salmo salar*, 17 days 22 hours, 10—12° C. Transversal section through the eye-muscle mesoderme and the first rudiment of the trabecle.

In a following stage (55 days 6 hours at 4° C.) (figs. 14, 15) the rudiment of the trabecula has grown considerably in length. The oral arch mesenchyme is attached to the trabecula frontally by means of a mesenchymatic body (int.b.). This relation will be discussed later on (p. 40). in this stage the polar cartilage (p.b.) has appeared as a diffuse mesenchymatic rod immediately behind the posterior end of the trabecula and scarcely separated from that structure. The polar cartilage rudiment is undoubtedly connected with the mandibular somite, from which it has probably arisen. It is not possible, however, to determine this derivation with absolute certainty, as the mandibular somite is but diffusely

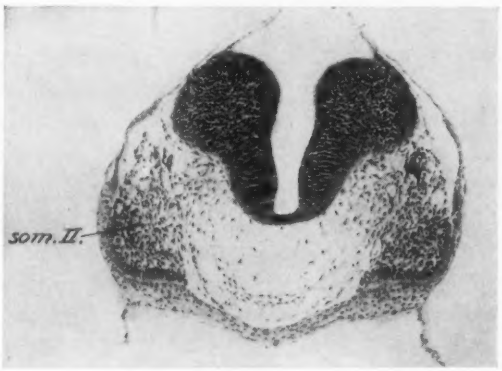


Fig. 11 b. *Salmo salar*, 19 days 8 hours, 10—12° C. Transversal section through the mandibular somite.

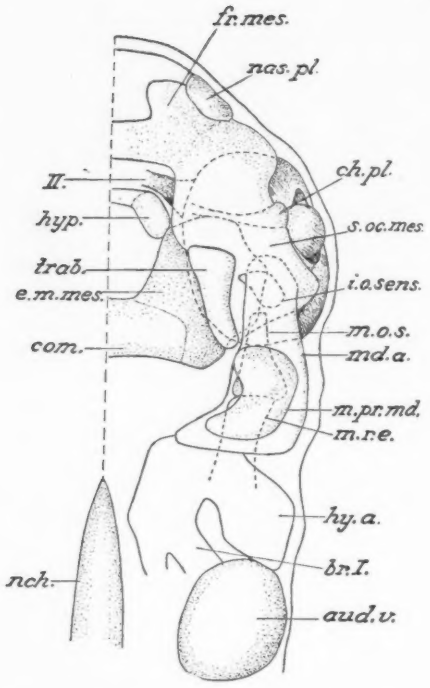


Fig. 12. *Salmo salar*, 48 days, 4° C. Reconstruction of the mesoderme of the head, ventral aspect.

outlined in this as well as in the preceding stages. In the subocular mesoderm the obliquus inferior muscle (m.o.i.) of the eye is about to develop myofibrillae. The frontal ento-mesoderm is now so mingled with ecto-mesoderm that a good outline could not be drawn. There thus exists a possibility that ecto-mesodermal cells could take part in the formation of the most anterior portion of the trabecular system. The eye-muscle-mesoderm has now become differentiated so that the rudiments of the musculus rectus superior (figs. 14, 15 m.r.s.), inferior (m.r.i.) and anterior (m.r.a.) are clearly determinable as processes issuing from a common centre on a level with the posterior part of the eye, where also the premandibular commissure (com.) is found (fig. 14). The common part of the eye-muscle-mesoderm has a high and broad medial surface which follows the side of the brain closely. The ectomesodermal oral arch mesenchyma has grown past the trabecula frontally and its lower jaw portion nearly reaches the ventral medial line of the head. It encloses the entomesodermal muscle process (m.pr.md.) of the mandibular somite. At the upper caudal end of the process a portion has partly detached and forms the rounded mesodermatic rudiment of the protractor muscle of the hyomandibula and of the operculum (fig. 14 m.pr.hm.). The rodlike rudiment of the upper obliquus muscle (m.o.s.) of the eye has now shifted far frontad above the eye. The rectus externus muscle (m.r.e.) is well developed as the frontal part of the difusedly outlined hyoid somite, which caudally is continued by the basiotic part of the auditory capsule. The basiotic lamina (fig. 14 lam.b.caps.) is frontally distant from the notochord; caudally it approaches it closely but does not come in contact with it. The (posterior?) parachordals (fig. 14 p.par.) are very short and lie on a level with a post-auditory part of the basiotic lamina. The auditory capsule consists of an anterior and a posterior auditory cartilage rudiment joined on to the basiotic lamina by means of an anterior and a posterior basiotic commissure respectively.

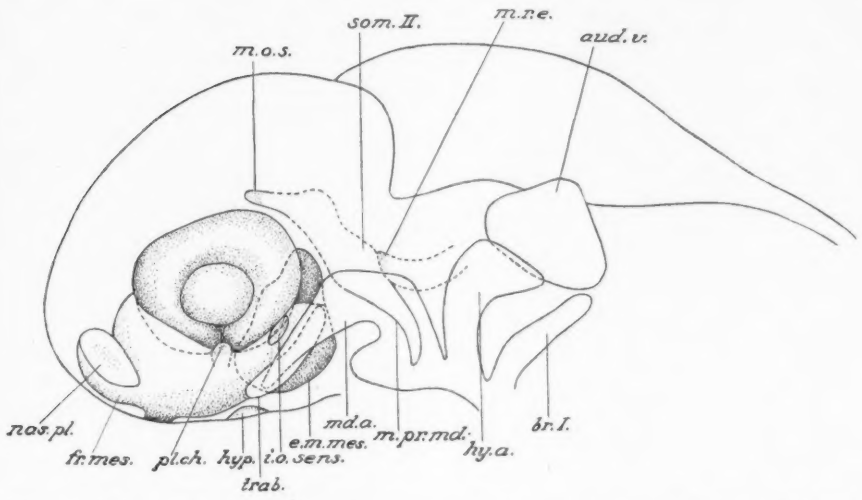


Fig. 13. *Salmo salar,* 48 days, 4° C. Reconstruction of the mesoderme of the head, lateral aspect.

The result of this investigation is that the trabecula is unquestionably of somitic origin, developing from the premandibular somite, and that the polar cartilage is probably derived from the mandibular somite.

The origin of the trabecula and of the polar cartilage in A c i p e n s e r.

The development of the trabecula of *Acipenser stellatus* has been studied by MATVEIEFF (1925). His treatment of the problem, however, seems to be too theoretical to allow any conclusion to be drawn concerning the early appearance of this structure. He begins his investigation with an 11 mm stage, where chondrification has already taken place in the trabecula. In this stage he finds a number of centres of chondrification and from this condition, and from his interpretation of the trabecula in Selachians, he draws certain conclusions, which unfortunately do not seem to have been evidenced by embryological studies of the actual development. According to MATVEIEFF the so-called trabecula and the supraorbital cartilage are the product of sclerotomes belonging to the premandibular and mandibular somites, of which the mandibular is said to have two and the premandibular one. As it is quite impossible at an 11 mm stage to find any genetical relation between the already strongly degenerated anterior head somites and the trabecula, MATVEIEFF's conclusion must be considered to be based on very little evidence. According to him, the thick anterior end of the parachordals are formed by an anterior sclerotome belonging to the hyoid somite. The trabecula and the supraorbital cartilage, he states, originate from the mandibular and premandibular somites and the

VOL. 24 1943

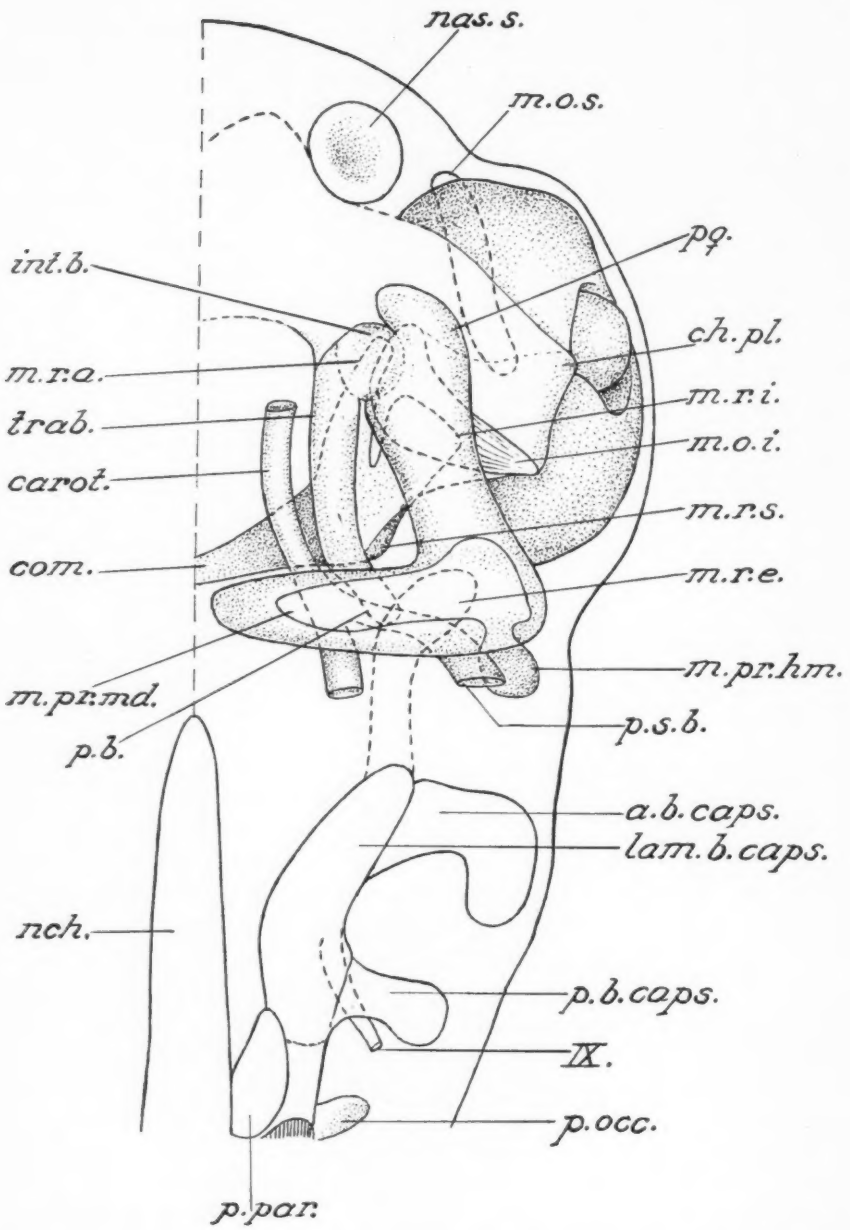


Fig. 14. *Salmo salar,* 55 days 6 hours, 4° C. Reconstruction of the mesoderme of the head, ventral aspect.

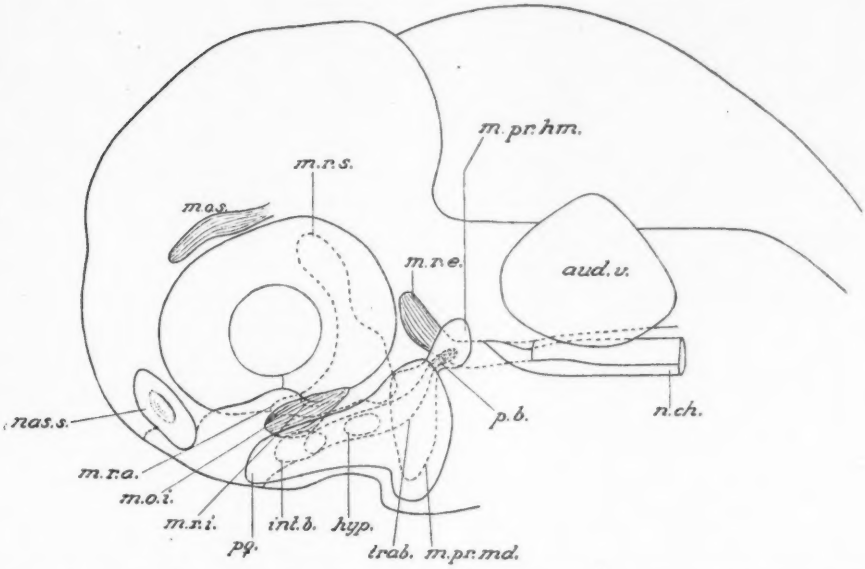


Fig. 15. *Salmo salar,* 55 days 6 hours, 4° C. Reconstruction of the mesoderme of the head, lateral aspect.

anterior end of the trabecula, which he considers to be the "trabecula" from mesenchyme, not of somitic origin.[1]

The reconstructions of the cranial skeleton in young stages of *Acipenser* published by DE BEER (1925), MATVEIEFF (1925) and SEWERTZOFF (1928) are all more or less erroneous, as the parachordals and trabeculae are represented as lying about on the same level. In reality there is a rather considerable angle between the anterior end of the parachordals and the trabeculae (polar cartilage and trabecula). At a 13 mm stage of *Acipenser stellatus* this angle is about 45° (fig. 27), at the 10 mm stage it is somewhat less (fig. 26). In addition there is a distinct frontal bend somewhat behind the middle of the trabecular complex, about at the boundary between the polar cartilage and the trabecular portion.

NEUMAYER (1932) has described an early part of the development of the head of *Acipenser sturio.* In this work he describes and figures the anterior (premandibular) head cavities in a 45-hour stage. They begin frontally on each side below the eyestalk and the eye. Behind the eye the inner lower margin of the two contralateral cavities or vesicles converge in a medio-caudal direction. This margin thereby becomes wedged in between the entoderm (archenteron) and the brain. Caudally (behind the future infundibulum) the

[1] MATVEIEFF has apparently been influenced by the interpretation of the trabecula of *Petromyzon* suggested by his master professor SEWERTZOFF.

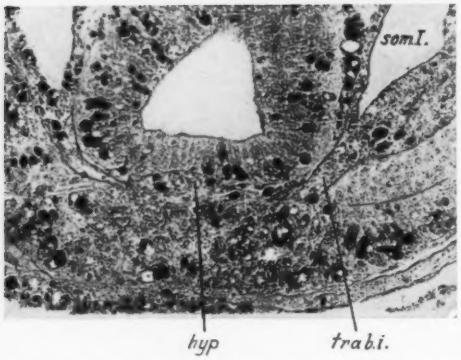


Fig. 16. *Acipenser ruthenus*, young embryo. Transversal section through the premandibular somite, with rudiment of the trabecle. Cartilage not yet developed.

vesicles are connected by a broad commissure. The rudiment of the inner carotid artery runs outside the ventral part of the premandibular cavity. In my material of *Acipenser ruthenus* there are some specimens showing about the same development as those of NEUMAYER, but in these the ventromedial border of the premandibular vesicle enters further mediad below the brain (fig. 16). During the subsequent development this border is withdrawn from the lower side of the brain so that the entire vesicle becomes lateral in relation to the brain (fig. 17). By this withdrawal, however, cells seem successively to be detached from the vesicle forming a strand of mesenchymatic cells along the course followed by the border of the vesicle (fig. 17). This strand of cells forms probably the earliest rudiment of the trabecula, viz. that part of the trabecula which lies inside the carotid artery.

The youngest rudiment of the trabecula of *Acipenser* described in literature is—as far as I know—that described and figured by SEWERTZOFF (1928). It belongs to a "very young larva" of *Acipenser ruthenus*. The rudiment is quite mesenchymatic. It is represented by a mesenchymatic bar, much thickened rostrally. Caudally the trabecular rudiment consists of a strip of rather loose mesenchyme, in which the polar cartilage is subsequently differentiated. This caudal portion adheres at the ventral part of the anterior end of the corresponding "parachordal" plate. At a somewhat later stage SEWERTZOFF demonstrates the carotid artery lying laterally to the trabecular rudiment. At a still later stage there is a broad incisure on the lateral basal part of the trabecula. Through this incisure the carotid artery passes dorsad. Later on this incisure becomes closed and there is thus formed a cartilaginous bar outside the carotid artery. Such a stage of the development of the trabecula is illustrated by MATVEIEFF (1925) and by HOLMGREN (HOLMGREN and STENSIÖ, 1936). As SEWERTZOFF paid no attention to the cephalic somites, he could not tell anything about the earliest development of the trabecula.

The earliest rudiment of the trabecula—derived from the premandibular somite—described in the preceding lines apparently corresponds to that part of the developed trabecula which lies inside the carotid artery. The part lying outside the artery is also a product of the activity of the premandibular somite. It develops later from a part of the somite situated on the lateral side

of its ventromedial edge. As seen in fig. 17, there is a centre from which cells wander out from the epithelial wall of the somite, moving apparently in a ventromedial direction outside the carotid artery. The ventromedial edge of the premandibular somite is now occupied by the rudiment of the obliquus inferior muscle of the eye.

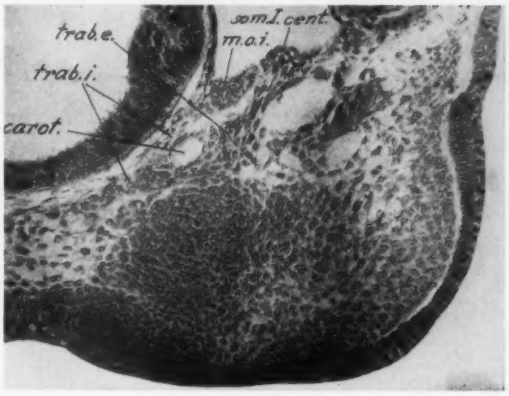


Fig. 17. *Acipenser ruthenus*. Embryo somewhat older than in fig. 16. External part of the trabecle developing.

At the stage when the carotid artery passes through a notch on the outside of the trabecula, that part of the trabecula which forms outside the artery is also present, though it is quite mesenchymatic. This mesenchymatic band, however, chondrifies much later than the medial part of the trabecula.

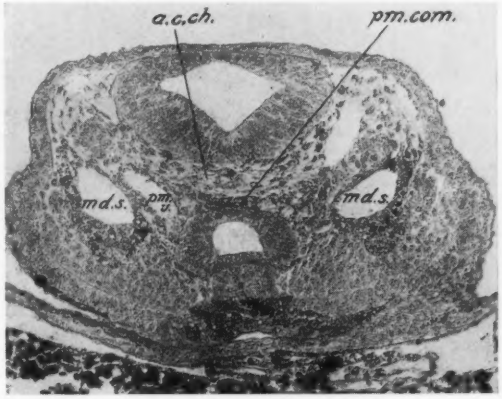


Fig. 18. *Acipenser ruthenus*, same series as fig. 16. Transverse section through the premandibular commissure.

The early development of the polar cartilage is still unknown, but MATVEIEFF has concluded that it must be derived from a part of the third cephalic somite, forming the most anterior thickened part of the "parachordals" of SEWERTZOFF's description. According to SEWERTZOFF, the narrow posterior part of the trabecular bar of his youngest stage represents the rudiment of the polar cartilage, whereas MATVEIEFF considers this part to belong to the trabecula proper (posterior centre of chondrification). My examination of a very large body of material of younger stages give results which do not tally with those recorded by either of these authors.

In a young egg stage the rudimentary brain flexure contains partly the thick premandibular commissure (fig. 18 pm.com.), partly cells detaching from the medial surface of the posterior part of the mandibular somite (fig. 18). In a somewhat later stage, at which the brain flexure is already well developed, the premandibular commissure is desintegrating, its cells interming-

VOL. 24 1943

ling with those derived from the mandibular somite. These cellmasses form the so-called acrochordal tissue (ac.ch) (the acrochordal tissue together with the polar cartilage).

In an 8 mm *Acipenser Gyldenstaedt* the mandibular somite is still a rather wide vesicle. Its wall consists of big yolk-laden cells. Cells are seen to become detached from the medial wall of the vesicle, filling up the space between the two contralateral vesicles. This space lies partly in the big brain flexure, partly behind the hypothalmus. A portion of these cells condense in front of, below and on the sides of the tip of the notochord.

In a 10 mm *Acipenser stellatus* the cells have decreased in size laterally and a little behind the hypothalmus. These smaller cells merge dorsally without any line of demarcation into de condensation at the anterior end of the notochord. This condensation chondrifies from behind and the chondrification gradually extends towards the region of the smaller cells. How far frontad this region reaches I could not determine with any certainty, but it seems possible that the anterior limit might lie at the locus of the future carotid incisure of the "trabecula". The main ventral portion of the anterior thickened end of the "parachordals" of SEWERTZOFF corresponds to the upper (caudal), at first chondrifying part of the rudiment just described, and his polar cartilages to the lower (anterior) part of it. MATVEIEFF calls the former part III,1S and the latter II,2S, thereby indicating that they should belong to different segments (hyoid and mandibular), which is not the case, as both arise from the mandibular (2d) somite of the head. As there is no line of demarcation between the two parts at any stage of development, they must be considered to belong to the same unit and together form the polar + the acrochordal cartilage.

The dorsal surface of the anterior thickened end of the "parachordals" of SEWERTZOFF is covered with the thin anterior ends of the true parachordals, probably derived from the hyoid somite.

The polar cartilage + the acrochordal derived from the mandibular somite, forms the ventral portion of the dorsum sellae, the dorsal of which is formed by the frontal end of the parachordals. The caudal part of the "trabecular bar" behind the carotid incisure probably also belongs to the polar cartilage. This incisure will be closed to a foramen later, when the lateral part of the trabecle is formed (Cfr. SEWERTZOFF). As that part of the trabecula which lies frontally to the carotid foramen is probably derived from the premandibular somite and the polar cartilage from the mandibular, the carotid foramen ought to lie on the boundary between these two constituents, almost where MATVIEFF draws the line of demarcation between his sclerotomes II,2S and II,1S.

The origin of the trabecula and of the polar cartilage in Amia.

At the earliest stage of development (7.5 mm) of *Amia*, hitherto investigated in regard to the development of the trabeculae, the polar cartilage is already fused on to the trabecula, which is chondrified or chondrifying (PEHRSON, 1922). Such a stage is therefore too far advanced for studying the initial stages of the development of these cranial parts.

My material of *Amia* does not allow of any close analysis of the provenience of the material of which the polar cartilage and the trabecula are formed. My sections of early stages indicate, however, that the polar cartilage may be formed from the mandibular somite. Nevertheless its post-carotid process may possibly have originated from the premandibular somite, as it forms close to its ventrocaudal border.

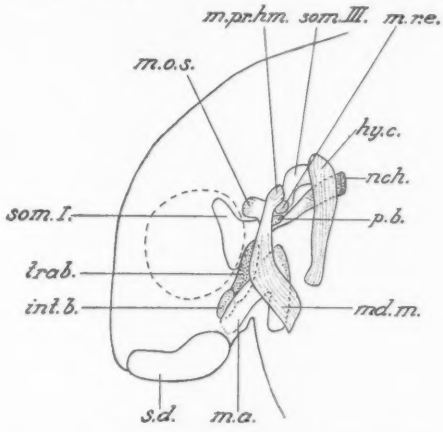


Fig. 19. *Amia calva.* Embryo 6 mm. Reconstruction of head structures, lateral aspect.

At a 6 mm stage (fig. 19) at my disposal the loci of the trabecula and the polar cartilage are discernible as rather diffuse portions of mesenchymatic tissue. Only the most anterior part of the trabecula is tolerably well delimited. This part corresponds to the thick blastematic portion of the trabecula illustrated by PEHRSON in an 8 mm stage. It is with this part the trabecular commissure will subsequently be connected. The rudiment of the polar cartilage lies inside the very reduced mandibular somite and below the rudiment of the rectus externus muscle of the eye. The carotid artery lies ventrally to the rudiment. The rudiment of the trabecula is quite free from the polar cartilage. The caudal portion of the former lies outside the postero-ventral portion of the premandibular somite and *outside* the carotid artery. A mesenchymatic lamella extends from the polar cartilage frontad internally to and below the posterior margin of the premandibular somite and inside the carotid artery. This lamella answers to the first trabecular rudiment in *Acipenser*. The frontal part of the trabecula lies ventro-laterally to the carotis interna and ventro-medially to the lower orbital sinus. The carotid artery goes through the angle formed by the trabecula and the above-mentioned lamella of the polar cartilage.

At an early 7.5 mm stage the blastemas of the polar cartilage and the trabecula are in process of fusing outside the carotid artery and the lower border of the inner lamella of the polar cartilage rudiment retires dorsad to form a

VOL. 24 1943

process on this cartilage inside and behind the carotid artery. To this process all the rectus muscles of the eye, except the rectus externus, converge to find their insertion upon this process somewhat later. At an older 7.5 mm stage the rudiments of the trabecula and the polar cartilage are in process of chondrifying, the anterior portion of the former, however, remaining blastematic. The process on the polar cartilage, earlier almost vertical, is now more horizontal, forming the well-known frontal inside-process on the polar cartilage, into which the eye-muscles are inserted. This process is the primary trabecula, homologous with the at first developing trabecular bar in *Acipenser*. The definitive trabecula of *Amia* represents that part of the trabecula of *Acipenser* which lies laterally to the carotid artery.

THE RELATIONS OF THE CAROTID, THE EFFERENT PSEUDOBRANCHIAL AND THE OPHTHALMIC ARTERIES TO THE POLAR CARTILAGE AND THE TRABECULA IN SELACHIANS AND TELEOSTOMIANS.

In this chapter I intend to discuss the problem of the different course of the carotid and the pseudobranchial artery in *Selachians* and *Teleostomians,* pointing out some circumstances which may lead to the solution of this problem.

It has been stated, for instance, by DE BEER (1924) "that in Selachians the efferent pseudobranchial vessel joins the internal carotid by passing over the trabecula, whereas in all other groups it passes under the trabecula". This statement is correct concerning the adult conditions, but in young embryos of *Acipenser* the pseudobranchial artery passes over the trabecula, or at least falls into the inner carotid at the point where this vessel is about to cross over the trabecula dorsally.

There are thus three different cases to account for concerning the relations between the arteries and the trabecular system, viz.

1. The Selachians, with a medial inner carotid and the pseudobranchial crossing the trabecula on its dorsal side.

2. *Acipenser* and *Polypterus* with the posterior part of the carotid artery external and the anterior part internal, crossing the trabecula on its dorsal side; the pseudobranchial artery not entering the skull, but joining the posterior part of the carotid artery. But if the artery were to slip frontad along the carotid it would enter the skull dorsally to the trabecula. *Polypterus* has no pseudobranchial artery.

3. The osteoichthyes and teleosts with the posterior part of the carotid artery external and the anterior part internal, crossing the trabecula on its

ventral side. The pseudobranchial artery joining the anterior part of the carotid artery, crossing the trabecula ventrally.[1]

In teleostomian fishes there thus occur two different principles concerning the course of the inner carotid and partly also of the pseudobranchial artery: 1. that of *Acipenser* and *Polypterus* with a dorsal carotis interna and 2. that of *Amia, Lepidosteus* and most teleosts with a ventral carotis interna. It seems unlikely that the carotis should have followed a different course in the two cases. Assuming that this is not the case, we must ask ourselves whether the trabecular system in the two groups shows corresponding development. The development of the polar cartilage and the trabecula has been described in the preceding pages in *Acipenser* and *Amia* (and *Salmo*). There it was demonstrated that the posterior part of the trabeculae of these two groups of ganoids has a different position in relation to the brain: in the very young *Acipenser* this part lying close to the brain, in *Amia* bending out considerably from it. This difference between the two trabecular systems has already been explained in the following way in the preceding pages:

1. In *Acipenser* there are two different connections between the trabecula and the polar cartilage, one medially and one laterally to the carotid artery. In *Acipenser* the medial (primary) chondrifies early and is generally considered to be the trabecula proper (Compare SEWERTZOFF, 1928).

2. In *Amia* there is but one complete connection between the trabecula and the polar cartilage. It lies outside the carotid artery. But there is also present a not fully developed rudiment of a medial connection. This latter forms the well-known medial process on the inside of the trabecular complex. In *Amia* the outside connection and the inside process chondrify. (Early embryos of *Corydoras* behave as *Amia*, possessing the inside process. In older embryos this process joins the trabecula in front of the carotid artery, this artery thus piercing the trabecula.) In other teleosts the inside process is absent.

3. Thus the trabeculae of *Acipenser* and *Amia* are not quite the same morphological units, those of *Amia* corresponding to the lateral part of the trabeculae in *Acipenser*. This difference explains the different behaviour of the carotid arteries in the two types.

In Selachians the carotids are unpaired caudally, the unpaired part in the embryo lying behind (below) the polar cartilages. When this unpaired part divides frontally into a right and a left carotid, these enter between the trabeculae and are joined by the pseudobranchial artery, which crosses the polar cartilage on its dorsal side.

The selachian condition resembles that in *Acipenser* in as much as the pseudobranchial artery runs dorsally to the trabecula but differs from it in

[1] In *Amiurus* the pseudobranchial artery is said to cross the trabecula dorsally. In *Corydoras* the carotid artery pierces the trabecula and the pseudobranchial artery enters the carotid behind this point. In young stages both lie ventrally to the trabecula (Compare below). Probably the same is the case in *Amiurus*.

VOL. 24 1943

that the carotids run inside the polar cartilages and the trabeculae, whereas in *Acipenser* they run outside them.

De Beer (1924) has made an attempt to explain the difference, assuming that the trabecula remained unchanged. His explanation is purely constructive and refers to the conditions in teleosts, where the trabecula is crossed dorsally by the arteria ophthalmica magna, ventrally by the pseudobranchial artery. In Selachians the ophthalmica magna is a branch of the dorsal pseudobranchial artery. De Beer has constructed a hypothetical intermediate condition (fig. 20), in which the ophthalmica magna and the pseudobranchial artery are connected before crossing the trabecula. The selachian condition would then arise from the disappearance of the ventral vessel medially to the point of connection. The starting-point of this explanation is the assump-

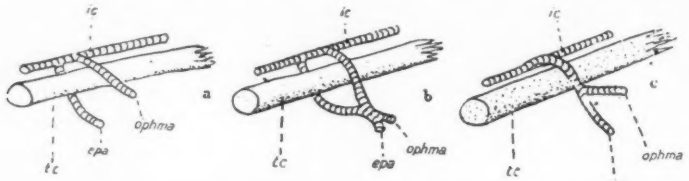


Fig. 20. Relations of the inner carotid, the ophthalmic and the efferent pseudobranchial arteries, a) in Selachians, c) in teleosts, b) hypothetical intermediary condition. Diagrams according to de Beer (1924).

tion that the selachian condition is secondary, because in all non-selachians the pseudobranchial artery "passes under that cartilage with constant regularity". De Beer (1937), however, says that the difference may depend upon "a change in the course of the bloodvessels, but in the absence of any evidence this view can hardly be regarded as morphologically satisfactory".

A very characteristic feature of Selachian embryos is the connection of the two inner carotids at the anterior end of the notochord. In the adult the connecting portion lies in the basis cranii. In teleosts a similar connection is common but, as will be elucidated below, it is of a secondary nature, whereas that of Selachians is probably primary.

In a 3.75 mm embryo of *Squalus* the angioblasts for the formation of the carotids are in process of separating from the medial border of the mandibular somites. (Compare Holmgren, 1940, p. 57). They consequently form strings, one on each side of the midline (rudiment of the subnotochord). Already at a 4 mm stage there are suggestions of a fusion of the strings frontally and at the 4.5 mm stage this fusion is very obvious at a point corresponding to the fused part at 5 mm and larger stages. Thus the fusion must probably be regarded as primary.

Ostroumoff (1908) found at an early stage of development of *Acipenser* before hatching, the two paired aortae in wide communication below the an-

terior end of the notochord. This communication frontally ceases somewhat behind the point where the efferent pseudobranchial artery falls into the carotid. I have established the correctness of this observation. In a 8 mm *Acipenser Gyldenstaedti* the unpaired vessel has become paired. There remain,

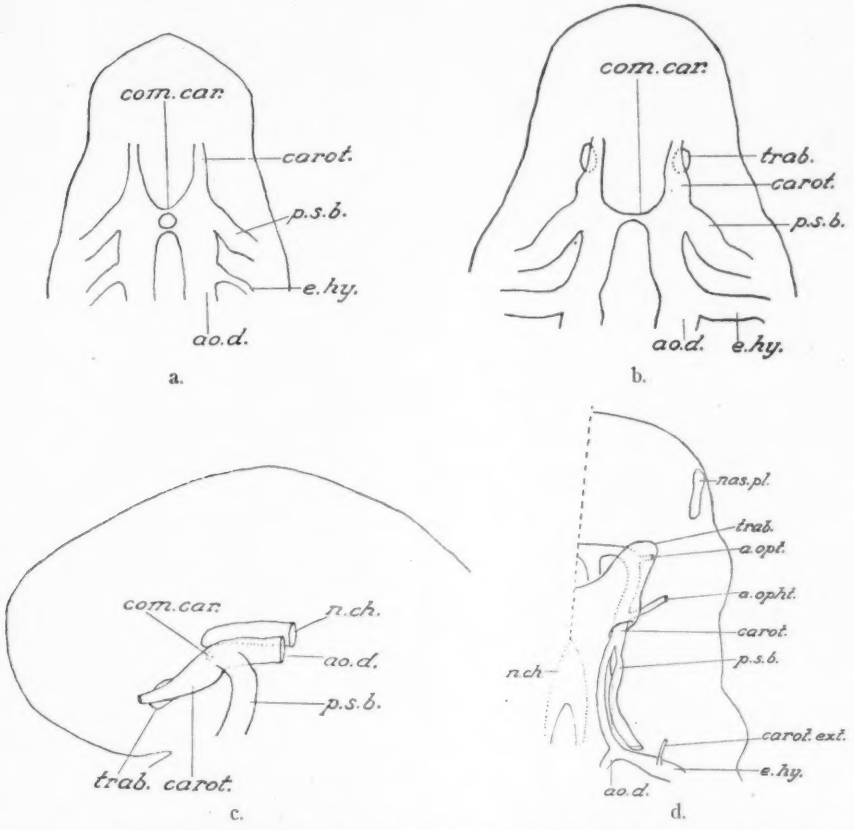


Fig. 21. *Acipenser Gyldenstaedti.* Three stages of the development of the arteries of the head a) 8 mm, b) and c) 9 mm, d) 14 mm stages.

however, two narrow commissural (fig. 21 a) vessels as rudiments of the earlier fusion. At a 9 mm stage (fig. 21 b, c) only one such rudiment is present as a strand of cells connecting the two carotids with one another. During this process of separation the carotids have successively absented themselves from the medial line. Later the carotids are quite free from one another and have come far apart (fig. 21 d).

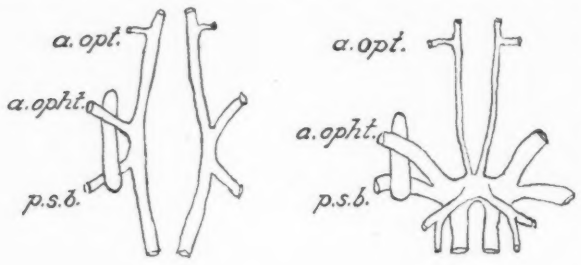
In Selachians and in *Acipenser* there is thus an early connection between the two carotids which is preserved in the former, but early breaks up in the latter.

VOL. 24 1943

In *Amia* and *Lepidosteus* the carotids are paired throughout the entire development.

In some teleosts, Siluroids (ALLIS, 1908), the carotids seem to behave as in *Amia,* but in others a connection between the carotids is present, for instance in *Salmo, Gadus* and *Esox* (ALLIS, 1912 c). I have studied the development of the carotis interna in *Salmo* and *Esox.*

In *Salmo salar* the carotids are independent of one another for a long time (fig. 22 a). The first stage at which fusion in the midline has taken place is in my material the 27 mm stage (fig. 22 b), i.e. a rather late stage of development. In *Salmo* the pseudobranchial artery (and the ophthalmica magna) is connected with the paired carotids already at a 19 mm stage at the point where later (27 mm stage) the carotid commissure will develop.



a. b.

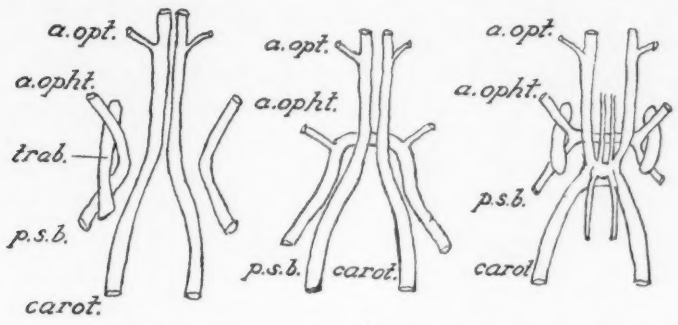
Fig. 22. *Salmo salar.* Two stages of the development of the arteries of the head a) 24 days stage, b) 27 mm stage.

In *Esox lucius* the carotids and the efferent pseudobranchial (and the ophthalmic) artery never (ALLIS, 1912 c) join. At a 9.8 mm stage (fig. 23 a) the two carotids lie paralell near the midline, and the efferent pseudobranchial artery bends dorsad inside the trabecula to turn outside it on its dorsal side, running straight for the eye as the ophthalmica magna. At a 10.6 mm *Esox* (fig. 23 b) a cross-commissure has developed between the two pseudobranchial arteries. This commissure runs ventrally to the carotids. At the 20.5 mm stage (fig. 23 c) the carotids have become joined by means of a commissure which lies behind the pseudobranchial artery.

The preceding discussion seems to confirm ALLIS' (1912 c) suggestion that in Selachians and *Acipenser* the carotid commissure is primary and that in teleosts secondary. There thus seem to be two kinds of *circulus cephalicus* present in fishes, a primary and a secondary.

(In *Ceratodus,* in which the inner carotid enters the brain cavity ventrally (medially) and the ophthalmica magna dorsally to the trabecula, there is present a primary circulus. The efferent pseudobranchial artery falls into the carotid inside the palato-quadrate.)

In the preceding lines I have given an explanation of the differences between the internal carotic system in *Acipenser* and in other teleostomians (*Amia,* teleosts). It now remains to try to explain the differences between *Acipenser* and the Selachians. The explanation offered by DE BEER (1924)



a. b. c.

Fig. 23. *Esox lucius.* Three stages of the development of the arteries of the head a) 9.8 mm, b) 10.6 mm, c) 20.5 mm stages.

has already been quoted (p. 24). It is purely constructive and therefore not satisfactory. A satisfactory explanation must be borne out by actual facts. In the first part of this paper (HOLMGREN, 1940) I have described the relation between the mandibular somite and the rudiment of the polar cartilage in a 16.5 mm *Squalus* embryo. I quote the following lines: "At the level of the hooklike tip of the notochord the mandibular vesicle has its greatest transversal extension. Here its ventral and ventromedial wall is thinned owing to outwandering cell material which is in process of forming a body of rather densely arranged mesenchymatic cells dorsally to the future pseudobranchial artery (fig. 26 p.s.b.)". (Compare fig. 24.) This body is the initial rudiment of the polar cartilage of the trabecular system. The lines quoted satisfactorily solve the problem of the course of the pseudobranchial artery in adult sharks: the pseudobranchial artery shifts during embryonic life from its primary ventral position to the definitive dorsal. An examination of series of embryos older than 16.5 mm shows that the artery moves dorsad in front of the polar cartilage, slipping through the space separating the polar cartilage from the trabecula.

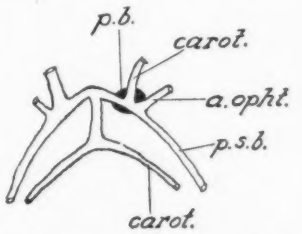


Fig. 24. *Squalus acanthias.* Embryo 16.5 mm. Relations of the ophtalmic and pseudobranchial arteries to the polar cartilage. *Ventral* aspect.

The preceding statement removes the last difficulty concerning the problem of the relations between the carotid, pseudobranchial and ophthalmic arteries and the trabecula. It is now possible to define the ancestral relationship of the said organs. In an ancestral fish the pseudobranchial artery runs ventrally to the trabecula and the polar cartilage. The inner carotids were probably connected medially by means of a commissure or were simply fused medially. The conditions in Selachians regarding the course of the pseudobranchial artery are thus secondary, as presumed by DE BEER (1924). Of living fishes *Ceratodus* has preserved the

ancestral condition with a circulus cephalicus and a ventral pseudobranchial artery.

The ophthalmic artery (mostly joined with the pseudobranchial artery) always runs dorsally to the trabecula, even when the pseudobranchial artery runs ventrally. In order to arrive at an explanation of this condition I have studied a number of developmental stages of *Salmo, Esox* and *Amia* and I have found that this artery develops at a stage where the trabecula is already developed (or developing). In *Salmo* the posterior part of the trabecula at that time lies close to the epithelium of the buccal cavity and the inner carotid artery immediately medially to the trabecula. At this point and a little frontally to the entrance of the efferent pseudobranchial artery in the carotid a small pocket is formed on the dorsal side of the carotid artery. This pocket later grows dorso-laterad directed toward the eye and becomes the ophthalmica magna. The ophthalmic artery is thus a secondary artery, independent of the changes of the carotid system. In *Salmo* its dorsal direction is dictated by the position of the trabecula in relation to the eye. The rudiment has to grow out to the eye by the only way that seems possible, viz. between the trabecula and the premandibular somite. The above quoted circumstances indicate that the course of the ophthalmic artery has very little bearing on the questions discussed.

The development of the ophthalmic artery in sharks (p. 121) differs so much from that in the teleosts that a doubt about its homology seems reasonable. On the other side, the anatomical behavior of the artery in both classes of fish is so similar that a doubt at the homology, seems to be excluded.

THE RELATIONS OF THE TRABECULAR BAR AND THE PALATO-QUADRATE IN SHARKS.

In the first part of this work (HOLMGREN, 1940) was described an early frontal connection between the palato-quadrate and the trabecula. This connection is effectuated between the first developing (epimandibular) part of the palato-quadrate and the so-called trabecular process (with the insertion of the obliquus inferior muscle) near at the frontal end of the trabecle). During the further development of this connection the so-called orbital articulation arose. The position of this articulation is variable in the adult, depending upon the mode of growth of the trabecula. In *Squalus* and *Etmopterus* the growth is chiefly located in a part of the trabecula lying frontally to the trabecular process. The result of this growth is that the articulation is located in the middle part of the orbit or behind that part. In *Scyllium* the growth of the trabecula is located in the portion behind the

trabecular process; the articulation thus became located in the anterior part of the orbit and in close connection with the orbitonasal plate. This connection between the trabecula and the palato-quadrate is so early that the palatine part of the latter has not yet developed. As is seen from the first part of this work (HOLMGREN, 1940) the palatine part differentiates into a mesenchymatic mass, of which the superficial part becomes the mesenchyme of the teeth, the deep part the palatine cartilage.

In addition to this frontal connection there is in shark embryos an incomplete basal connection in the region of the polar cartilage. In recent sharks this connection is never complete. In *Etmopterus* it is marked by the existence of a basitrabecular (basipolar) process from which issues a fibrous band connecting with the palato-quadrate. This band was shown to be the remnant of that mesodermal band which in early stages of development connects the rudiment of the polar cartilage with the remnant of the mandibular somite at the basis of its muscular process (HOLMGREN, 1940, figs. 34, 38).

VOL. 24 1943

THE RELATIONS OF THE TRABECULAR BAR AND THE PALATO-QUADRATE AND CERTAIN DERMAL BONES IN ACIPENSER.

SEWERTZOFF (1928) has given an account of a very early stage of development of the mandibular arch in *Acipenser ruthenus*. According to this description the not yet chondrifying mandibular arch is U-shaped and the palato-quadrate portion is not yet separated from the lower jaw portion. The rudiment of the mandibular musculature lies outside both portions and the mandibular branch of the trigeminal nerve outside the muscle rudiment.

SEWERTZOFF's description is, however, not complete, as will be demonstrated below.

In a 9 mm embryo of *Acipenser Gyldenstaedti* the mesenchyme and the musculature of the mandibular arch are about to differentiate. The trabeculae have not yet appeared, but the mesenchymatic tissue in which they will appear somewhat later (10 mm stage) is easily discernible at the ventromedial border of the carotid artery. The mesenchyme of the mandibular arch is well separated from the rudiment of the trabecula lying inside it, but the former is joined on to the latter by means of a body of mesenchyme that is not very clearly delimited. The point of junction is situated behind the frontal end of the trabecular mesenchyme. Frontally the mandibular arch mesenchyme runs without any demarcation into the mesenchyme of the future tentacles (which thus probably belong to the mandibular arch). The mandibular arch mesenchyme consists of two portions, a medial and a lateral. They are

separated from each other by the mandibular muscle process developed from the mandibular somite and piercing the mesenchyme mass (fig. 25). This mass is thus undivided in front of and behind the muscle rudiment. The medial part of the mesenchymatic mass is already at this stage discernible as the mandibular arch, with a dorsal palato-quadrate and a ventral mandibel portion. The future of the lateral mesenchyme can only be determined at later stages of development (13 mm stage of *A. stellatus*).

At 10 mm stages of *Acipenser stellatus* the palato-quadrate is in principle the same as in the 9 mm stage of *Gyldenstaedti,* but, the trabeculae + polar

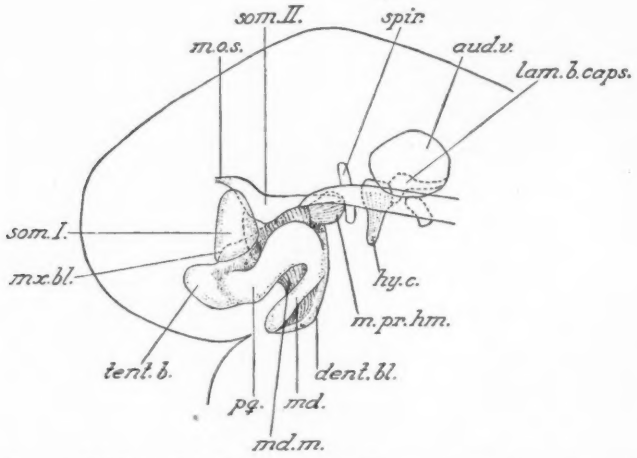


Fig. 25. *Acipenser Gyldenstaedti.* Embryo 9 mm. Reconstruction from the *medial* side.

cartilages are in process of forming (fig. 26). The anterior portion of the palato-quadrate mesenchyme is found as a condensation immediately below the trabecula, somewhat in front of its mid-point. At the most frontal end the palato-quadrate and the trabecular mesenchyme are joined together by the body (fig. 26 b) described in the 9 mm stage of *A. Gyldenstaedti.* This body is now better outlined than before.

At the 9 and 10 mm stages the position and relation of the palato-quadrate is the same as in other teleostomians and as in sharks, as it has not yet begun to shift away from the trabecula. At the 13 mm stage of *A. stellatus* its transformation is already so far advanced as to bring about the adult conditions in principle (fig. 27). Thus the anterior ends of the palato-quadrates have come quite close to one another in order ultimately to form a symphysis. They have also separated from the trabeculae. Through this separation, however, they have preserved their relation to the trabeculae, as that body, which at earlier stages connects with the trabecula, has been drawn out into a ligament (lig.), which in the adult connects the palato-quadrate with the

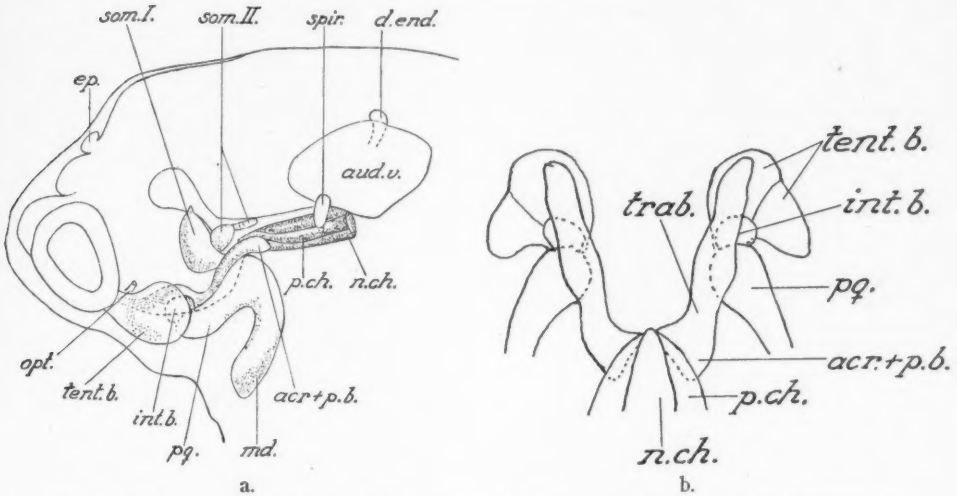


Fig. 26. *Acipenser stellatus.* Embryo 10 mm. a) Reconstruction of the head organs in lateral view, b) in dorsal view; relations of the trabecle, the palato-quadrate and the tentacle blastema.

neural skull (at the palatobasal process). Behind this ligament the connection is more or less lamelliform (lam.). The upper end of the posterior portion of the palato-quadrate rudiment behind the muscle rudiment and the mandibularis nerve as at the 9 mm stages of *A. stellatus* and *Gyldenstaedti* is not clearly demarcated but merges into a large mesenchyme field (spir.a.)

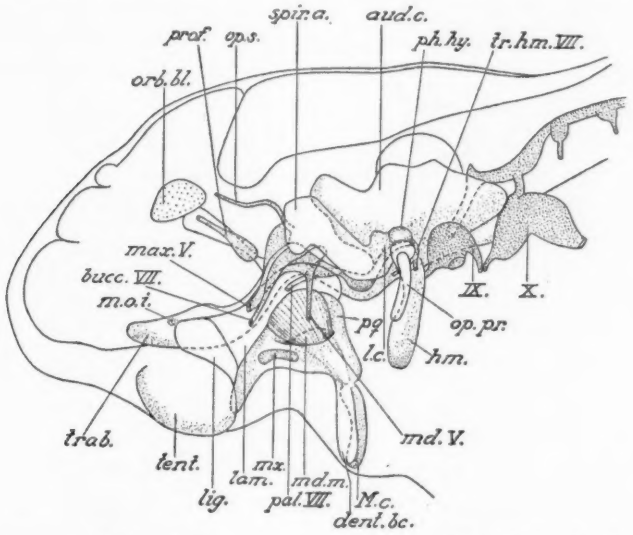


Fig. 27. *Acipenser stellatus.* Embryo 13 mm. Reconstruction of the head organs in lateral view.

extending backwards and upwards toward the spiracular canal rudiment. In this field is situated the rudiment of the protractor muscle of the hyomandibula (constrictor dorsalis of the mandibular arch). SEWERTZOFF has demonstrated that this muscle belongs to the mandibular musculature and is innervated from the trigeminal nerve. This muscle thus corresponds to the spiracular muscle (constrictor dorsalis of Selachians), which in selachians also has certain relations to the hyomandibula (*Torpedo*). It seems possible therefore that the said mesenchymatic field might include the never developing spiracular skeletal apparatus of *Acipenser*. Where, however, this mesenchymatic field (in a 13 mm embryo) comes into contact with the auditory capsule a membrane seems to differentiate, becoming attached to the rudiment of the postorbital process and the auditory capsule in front of the anterior border of the hyomandibula. This membrane (l.c.) lies outside the head vein and the facialis ganglion and must correspond in part to the lateral commissure of selachians. Somewhat later the membrane disappears, especially as the protractor muscle of the hyomandibula acquires a ventro-lateral insertion into the auditory capsule in front of the jugulo-hyomandibular canal and on both sides of the jugular vein. It must be strongly emphasized that the real evidence in which to base an interpretation of the described mesenchymatic field as belonging to the mandibular arch and the never developing prespiracular apparatus is constructive and can only be supported by a comparison with the selachian conditions. The real evidence is the early and transitorial existence of this dense mesenchyme, in which the spiracular apparatus must have been located, if it was really present at all.

In the 13 mm embryo the palato-quadrate has acquired its definitive shape (fig. 27). Now it is possible to establish that the medial part of the oral arch mesenchyma forms the palato-quadrate proper and the mandibula, whereas its lateral portion forms the rudiment of the future maxillare (mx.), the quadrato-jugale (dermoquadrate, SEWERTZOFF) and the dentale. The mandibular muscle thus lies inside and behind the maxillare, a fact to which SEWERTZOFF has attached great significance, as he considers it to be sufficient reason for regarding the maxillare as belonging to a reduced premandibular arch, homologous with the "posterior" pair of labial cartilages in Elasmobranchs.[1] I do not think that this justifies such an important conclusion. Nevertheless, I do not consider it excluded that SEWERTZOFF's interpretation may be correct, viz. for the following additional reasons. 1·0 The very close agreement between the *early* rudiment of the "posterior" labial cartilage of sharks with the early rudiment of the maxillare + dentale in *Acipenser*. The

[1] The "posterior" pair of labial cartilages in sharks is actually the anterior pair, as it develops in front of the "anterior". A shifting during the subsequent development is responsible for the real anterior cartilage becoming the "posterior" and *vice versa*. (HOLMGREN, 1940, 1942).

difference is that in *Acipenser* this rudiment is connected with the mandibular arch posteriorly also, which is not the case with the labial cartilage in sharks. This difference, however, may be correlated to the fact that in sharks the mandibular arch extends farther caudad than in *Acipenser*. 2:0 There is in *Acipenser*, at early stages, no frontal line of demarcation between the rudiment of the maxillare and the palato-quadrate. Moreover, the rudiments of the mandibula and the dentale are confluent frontally. 3:0 As late as the prochondrial stage there is no structural demarcation between the prochondrial portion of the palato-quadrate and mandibulare and the mass of cells in which the maxillare and the dentale respectively will appear. These matrix cells agree completely with common prochondrial cells.[1]

In conflict with SEWERTZOFF's hypothesis, however, stands the fact that the labial cartilages of sharks are true cartilages—the maxillare and the dentale of *Acipenser* membrane bones ("Deckknochen"). Such a difference is generally considered to justify the rejection of any attempt of a homologization. But cases in which chondrification takes place where ossification ought to occur are not unknown, even now. In rays, for instance, it is a fairly common occurence that envelopes of lateral line canals chondrify, whereas in Chimaeroids and teleostomians they give rise to dermal bones. In *Salmo*, on the other hand, it has been shown that a portion of the very rudiment from which part of the hyomandibula develops, also forms the matrix of the opercular bone (see p. 89).

Thus there seem to be good reasons in support of the view of SEWERTZOFF and I am inclined to accept it.

At the 10 mm stage of *Acipenser stellatus* another hitherto unknown connection occurs between the palato-quadrate and the anterior end of the "parachordals".

From the polar and from the acrochordal cartilages long collagenous fibres extend laterad to join the posterior upper margin of the palato-quadrate (basipolar or basitrabecular connection). This connection later takes part in the formation of the connective tissue lamella already mentioned in respect of the 13 mm stage. The basitrabecular connection in *Acipenser* is of great interest as it corresponds, partly at least, to a similar one already described in sharks (HOLMGREN, 1940). It has nothing to do with the palatobasal processes present in the adult *Acipenser* in front of the carotid foramen, nor with corresponding processes in sharks.

The palatobasal process develops on the trabecula at the point at which the primary connection between the trabecula and the palato-quadrate is located, i.e. behind the future ethmoidal plate and the ectethmoid (orbito-nasal lamina),

[1] This agreement may be responsible for the fact that the dentale of *Salmo* at its first appearance can be described as a perichondrial bone of the foremost part of the mandibular cartilage, whereas the later developed portion of the bone is a membrane bone.

VOL. 24 1943

at about a corresponding point where the orbital articulation becomes established in sharks, that also being the point of primary junction between the palato-quadrate and trabecular rudiments.

With regard to the connections between the palato-quadrate and the trabecular + polar cartilage complex, there seems to be substantial agreement between *Acipenser* and the selachians.

THE RELATION OF THE PALATO-QUADRATE TO THE TENTACLES IN ACIPENSER.

It was said with regard to the palato-quadrate of the 9 mm *Acipenser Gyldenstaedti* that its mesenchymatic rudiment runs without any line of demarcation into the mesenchyme of the future tentacles, which therefore probably belongs to the mandibular arch as its most anterior part. This latter conclusion, however, needs further elucidation. In a series of photographic reproductions of embryos of *Acipenser ruthenus* of different ages, SAVATSKY (1911) has demonstrated the mode of development of the tentacles. In his paper he has shown that their epithelial parts develop from the entodermal sucking organ of the young embryo.

The striking external resemblance between the mouth opening and its bordering structures in the young embryo of *Acipenser* and the corresponding parts in a young selachian embryo has given the idea that this resemblance might perhaps be of deeper significance than as a mere point of similarity. The head of a very young Acipenser is figured in fig. 28 a. At the frontal border of the mouth opening lie two thick swellings: the early rudiment of the tentacles. They contain the mesenchyma of the tentacles belonging to the mandibular arch. At a later stage each of these two swellings has divided into two: the rudiments of the future tentacles with their mesenchyma and cartilages. Fig. 28 b illustrates an embryo of *Torpedo*. Two swellings are also seen at the anterior border of the mouth opening. These contain the rudiment of the most anterior (palatine) part of the palato-quadrate. This part, as earlier described (HOLMGREN, 1940), develops much later than the posterior

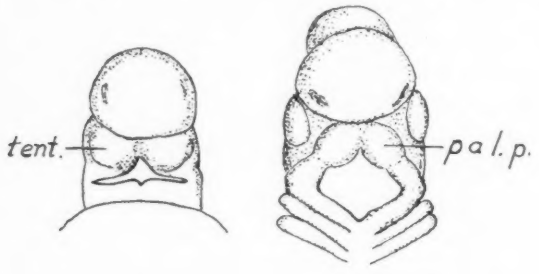


Fig. 28. a) Head of a 9 mm embryo of *Acipenser ruthenus*, ventral aspect. b) Head of an early embryo of *Torpedo ocellata*.

portion of the palato-quadrate, and in *Etmopterus* it chondrifies separately. During the actual *Torpedo* stage the swellings contain an undifferentiated mesenchyma which will subsequently differentiate into the fronto-medial portion of the palato-quadrate and the mesenchyma of the teeth. So far the comparison is possible, though somewhat superficial. An additional piece of evidence of greater importance, however, is offered by the fact that in both cases the palatinus VII nerve has its end branches in the mesenchyma of the compared parts. It may therefore be possible to assume that the tentacular mesenchymatic plate in *Acipenser* corresponds to the anteromesial portion of the palato-quadrate in selachians. This conclusion tallies with the fact that the palato-quadrate of *Acipenser* has no independently developed palatine part.

VOL. 24 1943

THE RELATIONS OF THE PALATO-QUADRATE AND THE TRABECULA AND CERTAIN DERMAL BONES IN AMIA.

At the 6 mm stage of *Amia* (fig. 19) the palato-quadrate part of the mandibular arch extends frontally to near the anterior tip of the trabecula, where it is connected with the latter by a short diffuse transversal mesenchymatic bridge. The mesenchyma of the mandibular arch is not clearly delimited dorso-caudally and seems to have a dorsal continuation in a diffuse mesenchymatic field in front of the spiracular rudiment and the rudiment of the dorsal constrictor muscle of the mandibular arch. This field corresponds to a similar field in *Acipenser,* where it was interpreted as representing never-developing prespiracular cartilage elements.

The mandibular arch mesenchyma is pierced by the broad muscular process of the mandibular somite, which divides it into a thinner lateral and a thicker medial portion. Frontally and caudally these portions are continuous. Immediately outside the mandibular muscle lies the mandibularis nerve.

At the early 7.5 mm stage, when the trabecula is traceable along its entire length, the blastema of the palato-quadrate behaves as in a 6 mm stage. As at the 6 mm stage, the anterior end portion is thicker than its more posterior parts (Cfr. PEHRSON, 1922). The frontal portion is separated from the epithelium by mesenchymatic cells forming a medial prolongation of the mesenchymatous cell mass developed between the trabecula and the anterior end of the palato-quadrate rudiment. On the prolongation a premaxillary tooth is developing and on the anterior part of the palato-quadrate rudiment a probably dermopalatine tooth is forming. A very young rudiment of a tooth lies immediately below the frontal end of the palato-quadrate blastema, behind its connection with the trabecula. This tooth is a pterygoid tooth.

At an 8 mm stage the conditions at the anterior ends of the trabecula and the palato-quadrate are more suitable for an analysis than in the preceding stage. Fig. 29 is a reproduction of a graphic reconstruction of the skull of an 8 mm *Amia* seen from the ventral side. The polar cartilage and the trabecula are in process of chondrifying. The most frontal part of the trabecula, however, is still blastematic. The trabecula, which lies outside the carotid artery, joins the outside of the polar cartilage, which has an anterior process inside the artery. The polar cartilage caudally merges without any demarcation into the preotic part of the basiotic cartilage. The parachordal, except for its most caudal portion, is still quite blastematic and forms a thickish band on the side of the notochord. In front of the anterior basicapsular commissure this band becomes thinner, less differentiated, forming a mesenchymatic membrane between the basiotic lamina + polar cartilage and the notochord. From this description it is clear that, as already was suggested by VAN WIJHE (1922), what earlier workers described as parachordals is the basiotic lamina.

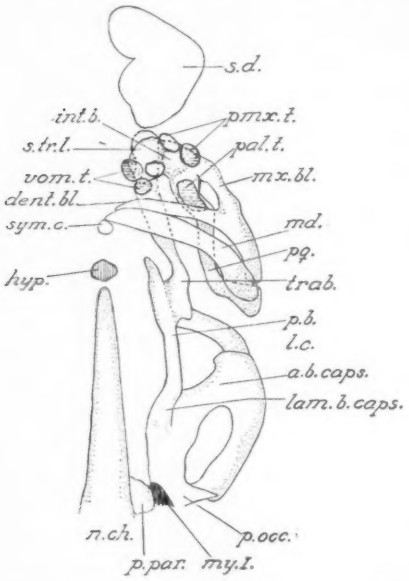


Fig. 29. *Amia calva*. Embryo 8 mm. Reconstruction of the right half of the skull, ventral aspect.

The trabecula ends frontally with a thickened blastematic portion or knob, into the lateral part of which is inserted the inferior obliquus muscle of the eye (fig. 30). Immediately behind and outside this knob there is a diffusely outlined rounded blastematic body connecting with the anterior end of the mandibular arch (fig. 29 int.b). The end portion of the trabecula is now separated from the epithelium. As a result of this separation either a ventrolateral part of the blastema became cut off from the trabecula proper, maintaining its position in contact with the epithelium, or else—as seems more probable—a lamella (s.tr.l.) belonging to the above-mentioned blastematic body (int.b.) pushed its way in below the tip of the trabecula. On the surface of the epithelium, corresponding to the blastematic body, a tooth rudiment is developing. This is the rudiment of a tooth on the future premaxillare (pmx.t.), which later develops into a separated ventral part of this body. The mesenchyma extends further laterad in front of the mandibular arch mesenchyma and here carries another (lateral) premaxillary tooth rudiment

(fig. 29). Corresponding to the subtrabecular lamella mentioned above there develops a group of three teeth. These are vomeral teeth (fig. 29 vom.t.).

As at the 7.5 mm stage, the frontal portion of the trabecular rudiment of the 8 mm stage is connected with the mesenchyma of the mandibular arch by means of a blastematic body. At a 9 mm stage the mentioned body is in process of chondrifying and fusing on to the outside of the trabecula. It forms the posterolateral corner of the future ethmoid plate.

Besides the anterior connection between the palato-quadrate and the trabecula there is a thin membrane connecting the two structures throughout their length. This membrane begins behind the anterior connection and ends on the trabecular side on a level with the polar cartilage and at the mandibular at the anterior process of a deep part of the palato-quadrate. The posterior border of the membrane is thickened and has some connection with the lateral commissure of the neural skull, but the nature of this connection could not be elucidated from my material of *Amia*. The thickened border of the said membrane is of special interest as it probably represents a basal connection (basitrabecular process) homologous with the basal process described by HAMMARBERG (1937) in *Lepidosteus* (12 mm). The long connecting membrane of *Amia* corresponds perfectly with a similar membrane which I have described (HOLMGREN, 1940) in sharks and rays.

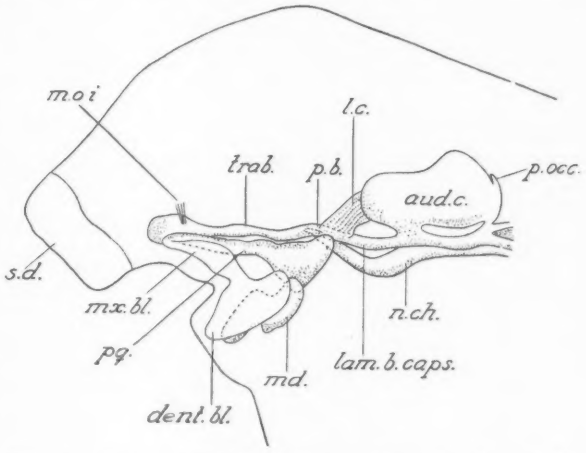


Fig. 30. *Amia calva*. Embryo 8 mm. Reconstruction of the skull, lateral aspect.

With regard to the earlier stages of development it was said that the muscular process of the mandibular somite pierces the mesenchyma of the mandibular arch, dividing it into a medial (palato-quadrate and mandibula) and a lateral subepithelial portion (fig. 30 mx.bl.). These two portions are continuous frontally and caudally. The antero-ventral portion of the palato-quadrate lies close to the epithelium of the border of the mouth. It is about to be divided into a superficial and a deep part. The latter is the autopalatine part of the palato-quadrate, the former the dermopalatine, which carries a tooth rudiment (fig. 29 pal.t.). But behind this anterior portion the palato-quadrate is also in contact with the epithelium of the mouth cavity. The cells that form

the contact surface are located at the ventrolateral margin of the prochondrial part of the palato-quadrate. They will later be cut off from the palato-quadrate to form the rudiment of the ectopterygoid. Mesenchymatic cells are also present at the ventromedial border of the palato-quadrate, connecting it with the mouth epithelium. These cells, however, are more scattered and apparently form a lateral part of a diffuse mesenchymatic membrane extending mediad toward the trabecula. At the 8 mm stage this membrane is not well defined, but at a 10 mm stage the membrane is very conspicuous. At later stages the entopterygoid develops partly (caudal part) on the epithelium side of this membrane and partly (anterior part) between the medial part of the palato-quadrate and the mouth epithelium. The said membrane is present along the palato-quadrate from its frontal part to the palatine nerve caudally, forming a longitudinal connection between the palato-quadrate and the trabecula.

The lateral portion of the mesenchyme of the oral arch forms a subepidermal layer outside the palato-quadrate (figs. 29, 30 mx.bl.). This layer could be followed caudad more or less parallel with the palato-quadrate, sending a broad branch round the corner of the mouth and out in the lower jaw parallel with the Meckelian cartilage (figs. 29, 30 dent.bl.). At the corner of the mouth this subepidermal layer is confluent with the rudiments of the palato-quadrate and the Meckelian cartilage. In elder stages (9, 9.5 mm) it is clearly seen that as in *Acipenser* part of the upper portion forms the rudiment of the maxillare and part of the lower gives rise to the dentale.

In front of the hypophysis the two trabeculae are joined by an intertrabecular mesenchyma including a part of the dura mater (8 mm stage). Frontally this "intertrabecula" later chondrifies, forming the so-called trabecular commissure. With increasing tropibasia this commissure grows longer and becomes compressed between the trabeculae and then incorporated with the "trabecula communis". On the buccal side of the "intertrabecula", between it and the mouth epithelium, a longitudinal band of mesenchymatic cells (superficial part of the medial area in sharks) is discernible. This band is the first appearing rudiment of the prehypophysial portion of the parasphenoid bone. At the frontal border of the hypophysis the mesenchymatic band divides into two branches passing along each side of the hypophysis and in contact with the mouth epithelium. Behind the dividing point the mesenchymatic band could only be followed for a short distance. At a 9.5 mm stage, however, the two branches were stated to follow the trabeculae and the polar cartilages past the hypophysis, to join on to an unpaired lamella underlying the parachordal portion of the basis cranii. In the mesenchymatic band the parasphenoid is already in process of forming in the 9.5 mm stage. In the region of the polar cartilages the rudiment of the parasphenoid has relation to the mesenchyma connecting the two polar cartilages. This relation here

corresponds to that between the intertrabecular tissue. But as far as I could find, the rudiment has no direct developmental relation to the trabeculae and the polar cartilages proper.

The parasphenoid bone in *Amia* has the wellknown parasphenoid wing or "processus ascendens", which lies outside the cartilaginous wall of the trigemino-facialis chamber (i.e. the lateral commissure). The paired aorta passes between the commissure and the ascending process. The nature of this process still remains unexplained. In an 11.5 mm embryo of *Amia* the rudiment of the ascending process is seen as a blastematic lamella close to the lateral commissure, between it and the spiracular canal. At a 12.5 mm stage the rudiment contains a bony lamella (Cfr. PEHRSON). The blastema ventromedially extends to the lateral margin of the paired aorta. The unpaired parasphenoid at the corresponding level is a broad unpaired bony lamella immediately below the endocranium, laterally bending down slightly toward the ventromedial margin of the paired aorta. At a 13.5 mm stage the conditions are almost the same, but the blastema of the processus ascendens seems to be about to join the blastematic border of the unpaired parasphenoid rudiment. At a 17 mm stage the parasphenoid bone has joined the processus ascendens bone and the parasphenoid has thus got its wings. For the independently formed bone on the lateral commissure, which will perhaps be found as a remaining independent bone in any extinct fish, I propose the name "lateral parasphenoid". This bone was described by PEHRSON (1940).

The "processus ascendens" of *Amia* must not be confused with the "processus ascendens" of *Acipenser*, the latter being a part of the true parasphenoid growing up on the sides of the endocranium, in close contact with it. In *Polypterus* probably both processes are present, the processus ascendens (of *Acipenser*) medially to the aorta and the lateral parasphenoid laterally to the aorta, forming the lateral wall of the "parasphenoid chamber" (ALLIS).

In *Lepidosteus* the unpaired bony rudiment of the parasphenoid bone at a 16.5 mm stage extends laterad to the aorta, but the blastema in which the bone has developed continues (without any bone lamella) on the ventral portion of the lateral commissure to the so-called palatobasal or basipterygial process, which according to HAMMARBERG is no such process, but a process on the lateral commissure. This lateral blastema lies outside the aorta. Ossification begins from the medial side and in continuity with the lateral border of the parasphenoid. Thus the lateral parasphenoid develops as a process of the parasphenoid proper in *Lepidosteus* and is never an independent element as in *Amia*. In *Lepidosteus* the relation between the palato-quadrate and the lateral commissure is very clear (HAMMARBERG). The relation between the lateral commissure and the lateral parasphenoid is also very conspicuous, as in the 16.5 mm and other young stages the blastema of this bone is not

VOL. 24 1943

possible to delimit structurally from the superficial portion of the lateral commissure.

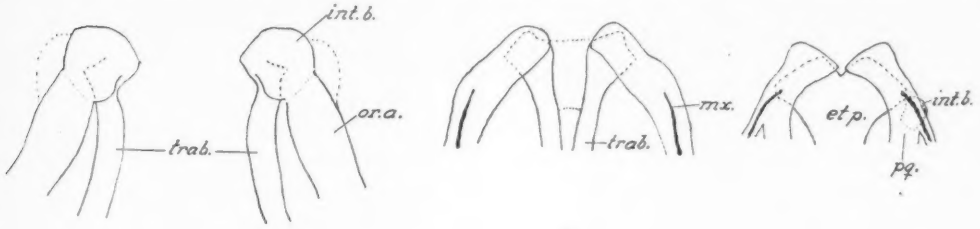
In the preceding pages it has been shown to be probable that the lateral commissure is a product of the mandibular arch. The development of a membrane bone on the commissure seems therefore to speak for the possibility that in this part of the arch also a deep part of the rudiment develops into cartilage, a more superficial into membrane bone, as is the case in the other portions of the mandibular arch and in the intertrabecular area.

In his very interesting paper on the development of dermal bones in the skull of *Amia calva*, PEHRSON (1940) has given a detailed account of the "bones not developed in connection with the sensory canal system". In this account he has described the development of maxillary, supramaxillary, ecto- and entopterygoid, dental, angular, prearticular and parasphenoid, which have their origin in different parts of the mesenchymatous tissue that lies in the wall of the mouth cavity. The origin of this mesenchyme has not been described. In the preceding pages it has been shown that this mesenchyme is the superficial layer of the undifferentiated mesenchyme, of which the deeper parts form the mandibular arch. Of this mesenchyme the deep parts chondrify, the superficial ossify. For the details of the development of the named dermal bones see PEHRSON's paper, which contains a very interesting and exhaustive exposition of this question.

THE RELATIONS OF THE TRABECULA AND THE PALATO-QUADRATE IN TELEOSTS.

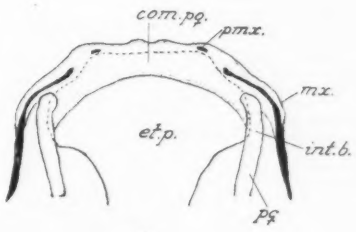
In the description of the development of the trabecula of *Salmo* given in preceding pages (p. 12) the early development of the palato-quadrate was also demonstrated (figs. 12—15). In the early stages there considered it was shown that there was originally no connection between the trabecula and the palato-quadrate but that such a connection became established by means of a blastematic body interposing between the anterior ends of the two structures. The provenience of this body could not be fully cleared up, but it seems most likely that it belongs to the oral arch mesenchyme extending mediad below the tip of the trabecula. This assumption rests upon the fact that the connection of this body with the mesenchyme of the oral arch is broader and more intimate than that with the trabecula. At a somewhat later stage of development (63 days after fertilization by 4° C.) the mesenchymatic body has partly been pushed ventrally towards the trabecula, partly embracing it on its lateral side. (Fig. 31 a.) The tip of the trabecula has an angular process on its lateral border. In front of this angle the tip of the trabecula and the body are continuous. The limit between the oral arch mesenchyme proper and

the mesenchymatic body is obsolete. At a later stage (41 days old, 10—12° C.) (fig. 31 b) the trabeculae have shifted towards the medial line of the head, and a trabecular commissure is developing between their anterior ends. The anterior ends of the trabeculae are in process of chondrifying and they are simultanously growing in a lateral direction, in all probability partly by means of their own activity but perhaps also by the addition of mesenchymatic cells derived from that part of the mesenchymatic body which covers the trabecular



a. b. c.

angle of the preceding stage. The mesenchymatic body of this stage is not separated from the oral arch mesenchyme. The latter is about to divide into a dorsomedial part—the palato-quadrate—and a ventrolateral—the blastema of the maxillary bone. At the next stage of development (52 days old, 10—12° C.) the ethmoid plate and a trabecula communis have formed (fig. 31 c). The anterior end of the oral arch blastema, including the mesenchymatic body, has anteriorly grown so far mediad that it has approached close to its *vis à vis*. It now covers the anterolateral border of the ethmoid plate as well as its lateral angle. The mesenchyme of the palato-quadrate has now separated from the mesenchyme of the maxillary bone excepting frontally, where the two mesenchymas are still continuous. At this stage a mesenchymatic band (fig. 31 c) is differentiating at the lateral angle of the ethmoid plate. This band connects with the palato-quadrate somewhat behind the level of the angle. This band is the rudiment of a ligament later connecting the palato-quadrate with the ethmoid plate. Between the two ends of this band will arise the articulation between the rudiment of the palato-quadrate and the ethmoid plate. During the subsequently development the medial ends of the anterior blastematic portion of the palato-quadrate rudiment meet in the medial line so that a commissural connection arises between the palato-quadrates at the frontal and ventral sides of the anterior border of the ethmoid plate



d.

Fig. 31. *Salmo salar*. Stages of the development of the connection between palato-quadrate and trabeculae. a) 63 days embryo, 4° C., b) 41 days embryo, 10—12° C., c) 52 days embryo, 10—12° C., d) 17.5 mm stage. Ventral aspect.

VOL. 24 1943

(fig. 31 d). In the anterior part of this commissure the rudiments of the premaxillary bone develop, whereas the posterior part of the commissure becomes incorporated much later and successively with the lower surface of the ethmoid plate. *Esox* and *Lepidosteus* behave as *Salmo*.

In *Corydoras* the development of the anterior end of the palato-quadrate and the trabecula is similar and a commissure is also developed there. But in *Corydoras* the anterior part of the palato-quadrate blastema also forms the cartilaginous rods in the centre of the maxillary tentacles (fig. 32).

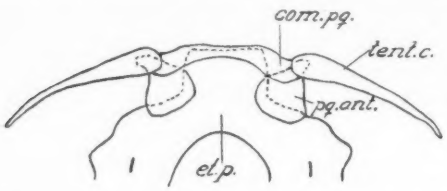


Fig. 32. *Corydoras palleatus*. Embryo 6 mm. Connection between palato-quadrate and trabeculae. Ventral aspect.

Cyclopterus is very interesting as the lateral parts of the commissure chondrify before fusing on to the ethmoidal plate (fig. 33), thus indicating that the commissure is a real, probably at one time a separate, skeletal element. In *Cyclopterus* the palato-quadrate cartilage frontally develops a process (pr.pq.) which passes considerably beyond the ethmoid region. This process is connected with the commissure by a blastema located on its medial side. At the stage illustrated in fig. 33 the big premaxillary bones have developed in the anterior part of the commissure, which has retained its blastematic character, whereas the posterior part has chondrified (comp.pq.). The maxillary blastema with the maxillary bone is well separated from the palato-quadrate.

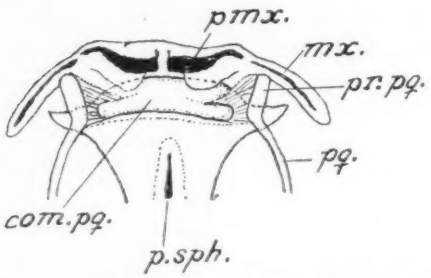


Fig. 33. *Cyclopterus lumpus*. Embryo two days after hatching. Connection between palato-quadrate and trabeculae, ventral aspect.

As in *Amia*, the palato-quadrate of *Salmo* is connected with the trabecula by means of a thin membrane along its upper border. The membrane ends caudally in the region of the polar cartilage and is there somewhat thickened. Caudally this thickened part—at a somewhat advanced stage continues dorsad in the form of a lamella constituting the anterior border of the lateral commissure which seems to arise through the chondrification of the lamella. The details of this process could not be studied in *Salmo*, where the outlines of the membranous parts are rather indistinct at earlier stages of development. In *Lepidosteus*, which behaves in the same way as *Amia* and *Salmo*, the following could be stated. At the 12 mm stage there is a basal connection between the palato-quadrate and the trabecula as HAMMARBERG (1937) has established and illustrated. This connection is thickened caudally at the palato-

quadrate side, and this thickened part, which is prochondrial, merges without any line of demarcation into the prochondrial anteroventral border of the developing lateral commmissure. There is thus some justification for the interpretation of the lateral commissure as a visceral structure. In sharks and rays I have furnished complete evidence to support the interpretation of the lateral commissure in those fishes as a differentiation of the spiracular skeletal apparatus, derived from the mandibular branchial ray bar. At the 16 mm stage of *Lepidosteus* the basal connection is preserved—contrary to the opinion of HAMMARBERG (1937)—but the palato-quadrate has acquired a second attachment to the skull by means of an articulation between the palato-quadrate and a strong process (parotic process) on the lateral commissure (HAMMARBERG, 1937).

VOL. 24 1943

COMPARISON AND CONCLUSION.

If we compare the relation between the trabecula and the palato-quadrate in the different fishes here considered, we invariably find that there is an early connection at their anterior ends. In *Scyllium canicula* and in *Salmo fario* this fact led DE BEER (1931 b) to believe that the trabecula arose from the mandibular arch and was thus a visceral structure, a theory refuted by the evidence that the trabecula is primarily an ento-mesodermal and the mandibular arch mesenchyme an ecto-mesodermal differentiation. From the fact that the two structures are of such fundamentally different origin the conclusion must be drawn that their early connection is a secondary one, a conclusion supported by real observations. Since the connection arose at the anterior ends of the two rudiments, there is so far nothing to justify the belief that this connection is different in sharks and teleosts. Accepting this standpoint we shall now proceed to try and make a closer comparison between the conditions obtaining in the two fish groups.

In adult sharks the early connection between the trabecula and the palato-quadrate lies in the orbital region, which is due to the growth of the trabecula. In Notidanids and Squaloids the location of the early connection has apparently shifted caudad, owing to the growth of the trabecula being chiefly located in the most anterior end of it. In galeoids the location is much more frontal, as the trabecula grows chiefly in its posterior part. Thus the locus of the early connection lies near the ethmoidal region in the Galeoids. In *Acipenser* the shark conditions are represented, but with this difference, that already at an early stage of development the trabecula has grown a rather long portion in front of the connecting point. In that fish this point becomes situated in the orbital region as in squaloid sharks.

The anterior end of the primary palato-quadrate of Selachians is bent-up towards the trabecula and this bent-up end (or process) forms the contact with the trabecula. Later this end forms the orbital process of the palato-quadrate and develops the strong ligament which in the adult joins the palato-quadrate with the neurocranium. In this ligament a chondrification has been described (SEWERTZOFF). In *Scyllium* the ligament develops earlier than the orbital process which forms at the palato-quadrate end of the ligament and secondarily fuses on to the palato-quadrate. In *Acipenser* the body which joins the palato-quadrate with the trabecula transforms into ligament in which chondrifications may develop. So far the Selachians and *Acipenser* agree. To the primary palato-quadrate of Selachians is added a palatine part which forms the palato-quadrate symphysial portion meeting its vis a vis in the medial line. This part originates independently and sometimes (*Etmopterus*) preserves its independence during chondrification, fusing later with the palato-quadrate proper (HOLMGREN, 1940). No such part is present in *Acipenser,* but it has been suggested in the preceding pages that the mesenchyme of the tentacles may represent this palatine part.

In *Amia* the conditions in the main resemble those in *Acipenser,* but the palato-quadrate joins the trabecula at its anterior end. The mediative body seems to develop a process below the anterior end of the trabecle. Judging from the development of the vomeral teeth below this process it may possibly represent, partly at least, a vomeral blastema. The conditions in *Amia* agree very closely with those of embryonic teleosts, in which the ethmoidal commissure has not jet been formed.

In *Salmo* and other teleosts the trabecular and palato-quadrate rudiments are joined anteriorly by means of a intermediary body. This body connects up partly with the lateral border, partly with the ventral surface of the ethmoidal region. In the lateral part will develop later the articulation between the palato-quadrate and the trabecula. In *Salmo* this articulation becomes divided into an anterior rostro-palatine and a posterior ethmo-palatine. In *Cyclopterus* and others the anterior articulation does not develop, the anterior end of the chondrified palato-quadrate extending frontad past the ethmoidal plate as a long rodlike process. The ethmo-palatine articulation is located immediately behind the ectethmoid process (orbito-nasal plate) which forms the caudal boundary of the ethmoidal region. This articulation may therefore be regarded as belonging to the orbital region of the skull and as thereby corresponding to the orbital articulation in sharks. The ligament which connects the ethmoid plate with the trabecula at the ethmo-palatine articulation thus corresponds to the orbital ligament in sharks.

As is seen from the description, the ventral part of the mediating body forms a commissure below the anterior border of the ethmoid plate. This commissure, which chondrifies almost separately in *Cyclopterus,* subsequently

fusing completely with the ethmoid plate, can scarcely be anything but the symphysial part of the palato-quadrate. As this part fuses early on to the ethmoid plate, it takes no part in the formation of the definitive palato-quadrate.

The following conclusion therefore is to be drawn from this comparison:

The palato-quadrate of the teleosts is built like that of sharks, consisting of 1) a palato-quadrate proper answering to the ontogenetically primary palato-quadrate in sharks, 2) an articulation ligament corresponding to the orbital ligament of sharks, 3) a symphysial (palatine) portion, fusing on to the lower surface of the ethmoid plate and corresponding to the palatine part of the palato-quadrate in sharks. In addition there is 4) a longitudinal membranous connection between the palato-quadrate and the trabecula and 5) in bony ganoids and teleosts a caudal thickening of this membrane, representing a basal process, present as a rudiment in sharks and in *Acipenser*.

VOL. 24 1943

THE MANDIBULAR AND HYOID ARCHES.

Observations on the branchial arches in Teleostomians and Selachians.

Any visceral arch theory relating tho the mandibular and hyoid arches must be based on some knowledge of the normal branchial arch. I shall therefore begin my account with a review of the anatomy and embryological development of the upper half of an anterior branchial arch in *Ganoids* (*Acipenser*) and *Selachians*.

In *Acipenser* each of the foremost interior branchial arches dorsally to the ceratobranchial elements consists of *three* cartilages, viz. the epibranchial, which carries the infra- and supra-pharyngobranchials. The infra-pharyngobranchial articulates with the medial part of the auditory capsule, the supra-pharyngobranchial against the lateral part of this capsule. Both lie medially to the head vein (vena capitis lateralis, jugular vein) as DE BEER (1925) has stated. (SCHMALHAUSEN (1923) has illustrated the vein between the two pharyngobranchials.) The efferent branchial artery passes between the two elements. The process of development of the branchial arches in *Acipenser* is but little known. DE BEER has found that at a 10.5 day stage of *Acipenser stellatus* the infra-pharyngobranchial "articulates directly with the ceratobranchial" and that "the epibranchial (supra-pharyngobranchial) does not separate them". The chain of branchial arch elements are according to DE BEER's statement: hypobranchial, ceratobranchial, infra-pharyngobranchial and supra-pharyngobranchial. In Selachians, in which only one ordinary separate pharyngobranchial is present, it follows dorsally to the epibranchial. It must therefore follow from the sequence of the branchial arch elements in

Acipenser that the infra-pharyngobranchial corresponds to at least part of an epibranchial, and the supra-pharyngobranchial to the pharyngobranchial in selachians (see also SEWERTZOFF, 1928, p. 51). A close examination of the embryological development of these elements will no doubt support this interpretation. (In *Polypterus* ALLIS (1918) has homologized the supra-pharyngobranchial with the epibranchial element of the arch.)

In an embryo of *Acipenser ruthenus* 7 days old (from fecundation) the rudiment of the first branchial arch is still undivided. It is represented by a somewhat curved rod of a dense blastema lying in close contact with the lining epithelium of the branchial cavity. From the upper end of this rod a stout process projects mediad between the epithelium of the branchial cavity and the horizontal part of the first efferent branchial artery. The head vein lies at some distance dorsally to the arch. In the space between the dorsal end of the arch and the vein a blastematic skeletal rudiment (fig. 34 s.ph.br.) is found in an 11 days old embryo, in which the arches are prochondrial. This rudiment is the now appearing supra-pharyngobranchial (found and illustrated by SEWERTZOFF, 1928). At this stage the branchial arch is in process of segmenting. The upper part of the bent principal rudiment has separated from the lower part, the latter being the ceratobranchial + hypobranchial. As at this stage there are but two consecutive elements present in the upper part, these must be the epibranchial and the pharyngobranchial. Of these the epibranchial carries the stout medial process already described. This process still shows no tendency to extend towards the otic capsule, but at a 25 days stage its medial end has grown so close to the otic capsule that its tip touches the lamina basicapsularis at the posterior border of the anterior basicapsular commissure. At a 37 days stage the process has become segmented off from the epibranchial (fig. 35), forming the infra-pharyngobranchial (i.ph.br.). During this stage the pharyngobranchial (supra-pharyngobranchial) has chondrified and gradually made close contact with the inside of the jugular vein, becoming articulated with the auditory capsule medially to the vein.

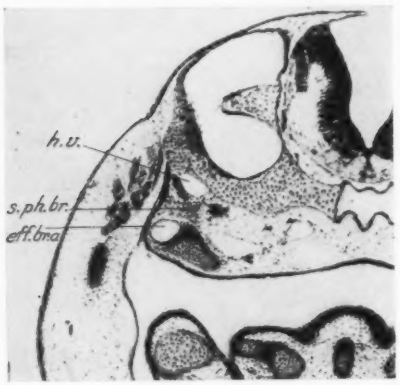


Fig. 34. *Acipenser ruthenus*. Embryo about 12 mm. Transversal section through the level of the first branchial arch.

In *Amia* and in *Lepidosteus* (HAMMARBERG, 1937) the infrapharyngobranchials develop from independent rudiments at the lining epithelium of the branchial cavity, whereas the suprapharyngobranchials[1] take their origin from

[1] In *Amia* considered by ALLIS 1897 to be the infrapharyngobranchial.

an epibranchial process lying posteriorly to the efferent branchial artery. As will be explained later, the difference between *Amia* and *Lepidosteus* and *Acipenser* is of no great consequence.

In *Polypterus* supra- as well as infra-pharyngobranchials are present, but neither of them seems to be completely segmented off from the epibranchial. The ontogenetical development, however, is still unknown.

As infra- and supra-pharyngobranchials are present in all living forms of ganoid fishes, it is clearly understood that these elements must be regarded as ancestral in the group of ganoids. There must have been present in any common ancestral fish type some structure which has been their predecessor. That the simple pharyngobranchial in Selachians corresponds to the suprapharyngobranchial in the ganoids needs no explanation. But the infrapharyngobranchials also seem to have a representative in the selachians. In embryos of *Squalus. Etmopterus, Scyllium, Heterodontus, Raja, Torpedo* and *Urolophus* (HOLMGREN, 1940) the upper end of at least the anterior epibranchials projects anteriorly in a more or less extended process lying below and anterior to the efferent branchial artery. At the tip of this process is inserted the interarcual ligament, extending from it to the basal part of the pharyngobranchial of the preceding arch. In *Torpedo* this ligament chondrifies together with the process and becomes an interarcual. In *Chimaera* and *Callorhynchus* also the epibranchials have a similar process in at least the three anterior branchial arches.

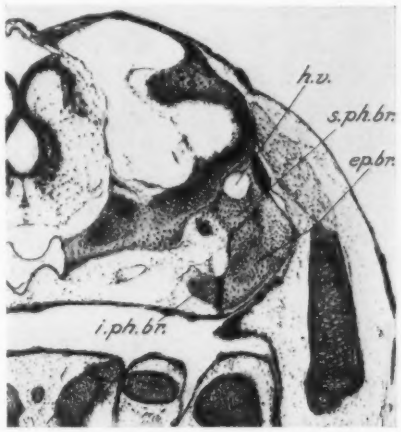


Fig. 35. *Acipenser ruthenus.* Embryo about 13 mm. Transversal section through the level of the first branchial arch.

A very striking correspondence must be considered to exist between the branchial arch of *Acipenser* 11 days old (see p. 46) and the normal selachian arch. As has already been pointed out, *Amia* and *Lepidosteus* behave somewhat differently in the development of their branchial arches, as the infrapharyngobranchial seems to develop independently at first, and the suprapharyngobranchials "grow out" of the epibranchial part of the arch. It was said that this is a difference of no great consequence. The reason for that opinion lies in the conditions in selachians. In the first part of this work (HOLMGREN, 1940) it has been shown that the first development of the branchial arches in selachians is different in sharks and rays. In sharks the first rudiments of the branchial arches consist of three separate mesen-

VOL. 24 1943

chymatic condensations, one for the (supra-)pharyngobranchial, one for the epibranchial (+ infra-pharyngobranchial) and one for the cerato- + hypobranchial. In rays there are but two rudiments: one dorsal and one ventral. From the dorsal the supra-pharyngobranchial grows out as a dorso-caudally directed process. The separate rudiments of sharks as well as of rays later fuse into one piece, which thereafter segments again into the different branchial elements, in rays the (supra-)pharyngobranchial thereby becoming a separate element. *Acipenser* thus behaves like the sharks, *Amia* and *Lepidosteus* like the rays in regard to the development of the supra-pharyngobranchial. Interarcuals may also develop as separate chondrifications in the interarcual ligaments, as is the case with the first interarcual, for instance, in *Urolophus*. Such an interarcual corresponds, partly at least, to an infra-pharyngobranchial.

When considering the palato-quadrate problem it must thus be borne in mind that there is strong evidence to prove the presence of an infra-pharyngobranchial element in the branchial arches.

In the branchial arches of selachians, however, two more elements must be named, viz. the extrabranchalia and the extraseptalia.

In connection with his hyomandibular theories ALLIS (1915) paid much attention to the extrabranchials. Some of the most important statements made by ALLIS about these cartilages, however, do not correspond closely to real conditions.

The extrabranchials are chondrified elements, considered to be specially modified dorsal and ventral branchial rays, differing from the other rays in their attachement to the pharyngeal or the upper end of the epal and to the hypal or the lower end of the ceratal cartilages respectively. If directly attached, as in rays, the are always attached to those elements of the same arch in the diaphragm of which they lie. If they are not directly attached, as in sharks, there is no reason to believe that they are not situated from beginning to end within their own arch. This is a condition that I have invariably found in all selachians investigated by me. DANIEL's (1922) statements also indicate the same view. When ALLIS says that "the conditions in *Laemargus* definitely show that the extrabranchials can come into contact with some element of the inner cartilaginous bar of the arch next posterior to the one which they belong", this statement must be rejected as inconsistent with facts, as in a *Somniosus* (*Laemargus*) at my disposal there is: 1) no contact between the extrabranchial and the inner cartilaginous bar (the branchial arch skeleton), and 2) the extrabranchial throughout its entire extension lies in one arch. Therefore the conditions in *Somniosus* are very little adapted for use as a guide to such an understanding of the principles of the extrabranchial relations. The only case in which I have found any relation between the extrabranchial of one arch and another arch is in *Torpedo,* where the extrabranchial normally

develops in its own arch but sends over a cartilagineous band to unite the epibranchial of the following arch.

In those selachians whose extrabranchial is attached to the upper part of its arch this attachement may be somewhat different. In *Urolophus* it is upon the pharyngo- and hypobranchial elements respectively, in *Torpedo* upon the epibranchial and ceratobranchial respectively. But in *Torpedo* the pad of connective tissue ontogenetically forming the connection between the upper extrabranchial and the epibranchial also extends over to the pharyngobranchial.

Other relations of the extrabranchials are those with the branchial rays of its own arch. Especially the upper extrabranchials are in many selachians connected with the tips of upper branchial rays. This is the case in at least one of the anterior arches in *Scyllium* and all in *Torpedo,* where extrabranchials are present.

VOL. 24 1943

The extrabranchials are said to be specialized in protective relation to the branchial portion of the jugular vein, as the upper cartilages in *Mustelus* lie "directly against the dorso-lateral portion of the large venous sinus". The ventral extrabranchials are said to lie ventro-mesial to the vena jugularis inferior. "The vein here lies between the base of the extrabranchial and the base of the adjacent ventral ray of the branchial series, definitely separating the one from the other". In *Heptanchus cinereus* and *Acanthias blainvillei* according to ALLIS the dorsal extrabranchials lie as in *Mustelus,* but in *Heterodontus (Cestracion)* "the dorsal ends of the dorsal extrabranchials pass dorso-mesial to the vena jugularis and almost reach the pharyngobranchials mesial to that vein". The relation of the dorsal extrabranchials of *Heterodontus* to the vena jugularis is thus said to be similar to that of the ventral extrabranchials of *Mustelus* to the vena jugularis inferior.

The morphological relation between the extrabranchials and the jugular vein is, however, as follows: In all cases where the extrabranchials are attached to branchial arches, and where their morphological relation could accurately be determined, they lie inside the vein. The dorsal extrabranchial thus lies morphologically ventrally to the jugular vein; the ventral extrabranchial dorsally to it. In those cases where no attachement is developed, the basal end of the dorsal extrabranchial lies ontogenetically inside the vein, i.e. ventrally to the vein, and the basal end of the ventral extrabranchial dorsally to the vena jugularis inferior. If there is any protective relation between extrabranchials and veins, this must be of a somewhat casual nature.

ALLIS concludes from his description of the relations of the upper extrabranchials that they are homologous with the suprapharyngobranchials of ganoid fishes. He says: "The relations of the suprapharyngobranchials to the vena jugularis are not given either by VAN WIJHE or BRIDGE, but it would seem as if they must lie lateral to that vein. If so they are certainly extrabranchials" ... In reality, however, the suprapharyngobranchials lie medial

to the vein, and therefore the conclusions founded upon the misinterpreted relation must be doubtful. In the preceding pages reasons have been given which make it extremely probable that the supra-pharyngobranchials of the ganoids correspond to the pharyngobranchials in selachians and that the infra-pharyngobranchials of Ganoids are represented by interarcual cartilages in selachians. The extrabranchials have no corresponding parts in Ganoids.

The extraseptalia described by M. FÜRBRINGER (1903) and mentioned by DANIEL in different rays as in *Torpedo, Raja, Myliobatis* and *Cephaloptera,* in *Torpedo* seem to belong ontogenetically to the tips of the branchial rays. These end with a chondrified lamella bent at about right angles to the rest of the ray and covering the outside of each branchial septum. These lamellae probably have a protective relation to the gills against pressure of the muscles of the pectoral fin and the electric organ.

Summing up, the structures and relations of a selachian branchial arch are the following:

1. The lower part of the branchial arch consists of:
   a. The hypobranchial, to which an extrabranchial can be attached, probably as a specially developed branchial ray.
   b. The ceratobranchial with a number of branchial rays, of which the most ventral perhaps represents an extrabranchial.
   c. The ventral extrabranchial in sharks is not attached to the inner branchial bar; in rays it is sometimes attached to the hypobranchial or to the lower end of the ceratobranchial.
   d. Branchial rays on the ceratobranchials and sometimes so-called extraseptalia on the tips of the rays.
2. The upper part of the branchial arch consists of:
   a. The epibranchial (with its upper anterior interarcual process or cartilage, infra-pharyngobranchial), with a number of branchial rays of which the most dorsal perhaps represents an extrabranchial.
   b. The pharyngobranchial (supra-pharyngobranchial), to which an extrabranchial can be attached, as a specially developed branchial ray.
   c. The dorsal extrabranchial is not attached in sharks. In rays it is attached to the suprapharyngobranchial or to the upper end of the epibranchial.
   d. Branchial rays and sometimes so-called extraseptalia on their tips.
3. Relations to some other structures:
   a. The efferent branchial artery passes anteriorly to the supra-pharyngeal (pharyngeal) cartilage, between it and the infra-pharyngeal process (interarcual) of the epibranchial.
   b. The pharyngeal elements lie ventro-mesially to the branchial portion of the dorsal jugular vein.

c. The dorsal extrabranchial elements morphologically lie ventrolaterally to the dorsal jugular vein.
d. The ventral extrabranchials morphologically lie dorsally or laterally to the "ventral jugular vein".

The morphology of the mandibular arch in sharks and rays.

The discussion of the general composition of the mandibular arch in Selachians has been principally concentrated upon the question of its homologies with the different parts of a normal branchial arch. Generally accepting the view that the palato-quadrate is the epal and the mechelian cartilage the ceratal part of the arch, the authors have occupied themselves in searching for the pharyngeal and hypal elements, which should make the homologies between the mandibular and the branchial arches more complete.

VOL. 24 1943

The "hypomandibula", according to DE BEER, should be present in *Heptanchus* and *Somniosus* (*Laemargus*) (WHITE, 1896) and in *Hexanchus* (K. FÜRBRINGER, (1903)) and SEWERTZOFF (1927). These records, however, are not quite correct, as WHITE found the "hypomandibular" in *Hexanchus* and *Somniosus* and FÜRBRINGER in *Somniosus* and *Chlamydoselachus*, where ALLIS (1923) did not find it. FÜRBRINGER failed to find this cartilage in *Heptanchus, Scymus, Etmopterus* (*Spinax*), *Centrophorus, Squalus* (*Acanthias*), *Centrina* and *Echinorhinus*. As GEGENBAUR did not find any "hypomandibular" in a single one of all the genera investigated by him, the occurence of the "hypomandibular" should properly be regarded as very occasional. It only seems to have been recorded as a diminutive cartilage nodule in the symphysis between the anterior ends of the mechelian cartilages in *Hexanchus, Chlamydoselachus* and *Somniosus*. In adult selachians I have seen this cartilage in *Somniosus* only, where it lies in the posterior part of the tough symphysial tissue as a chondrified portion of it. The symphysial tissue always is present in embryos and one or two condensed portions are regularly present in it. In *Heptanchus* an unpaired condensed portion is present in an embryo of 23 mm body-length. In a 75 mm embryo the symphysial tissue is in process of chondrifying dorso-frontally, there connecting the two lower jaws with each other. In a *Chlamydoselachus* of 127 mm there is present an unpaired rudiment, which may give rise to the symphysial cartilage observed by FÜRBRINGER. In *Heterodontus* and *Scyllium* one rudiment is also present, which chondrifies and joins the tips of the lower jaws. In *Squalus* at the 52 mm stage a cartilaginous nucleus lies in the symphysial tissue in front of and below the jaws. In *Etmopterus* (43 mm) a paired rudiment is present in addition to the unpaired symphysial tissue lamella. In *Torpedo* and *Raja* the symphysial tissue does not seem to chondrify but to form a tendinous mass between the lower jaws; in *Torpedo*

a medial slit developing in this mass forms an articular cavity. In *Urolophus* also a symphysial tissue lamella is present. At the 27—33 mm stages the frontal part of the lower jaws is prochondrial owing to the frontal growth of the jaws. The same seems to be the case in *Squalus*. From this survey of my material it is fairly obvious 1. that a symphysial tissue is always present in the embryos, 2. that this tissue is apt to chondrify and 3. that there may be two different elements present in the symphysis, viz. an unpaired medial and a paired lateral.

In *Etmopterus*, where the lateral elements are present in the embryos as well delimited prochondrial pieces, these elements fuse with the anterior ends of the lower jaws. From a similar condition, which I consider to be the primitive one, that of *Squalus* might be thought to have arisen through early fusion between the paired elements and the jaws. Such a fusion would explain the presence of the prochondrial anterior end of the jaw in this species and also in *Urolophus*. The unpaired symphysial element in *Squalus* is quite analogus to the symphysial element of the hyoid arch, viz. the basihyal.

In the preceding lines evidence seems to have been given for a theory concerning the morphology of the lower jaw of the selachians, according to which it consists of a cerato-mandibular on to the anterior end of which a small hypomandibular (paired rudiment in *Etmopterus*) has fused. A basimandibular is represented by the symphysial tissue, with or without an unpaired chondrification.

VAN WIJHE's (1922) statement that the lower jaw of *Squalus acanthias* chondrifies from two centres, affords strong support for the theory given in the preceding lines. The posterior of the two centres is greater than the anterior. VAN WIJHE compares these segments with the two parts of the mandibula, demonstrated by REIS (1896) in *Acanthodes Bronni*. REIS named the anterior segment the "prämandibulare". The "prämandibulare" in *Acanthodes* and the anterior centre of the mandible in *Squalus* and the paired rudiments in *Etmopterus* correspond far more closely to a hypomandibular than the unpaired symphysial cartilage of, for instance, *Somniosus*. In *Acanthodes* also an unpaired medial piece was present (REIS). See HOLMGREN, 1942.

In an *Arthrodiran, Leiosteus*, the lower jaw, according to STENSIÖ (1934) consisted of a posterior and an anterior paired ossified portion and an anterior symphysial unpaired part. The two paired portions must have been connected by cartilage. Presuming that the two centres of ossification of each half of the jaw also signify—as seems probable—two earlier cartilaginous centres[1], the jaw type of *Leiosteus* would thus correspond closely to the conditions in a selachian ancestor.

[1] In amphibians a corresponding composition of the embryonic jaw is well known.

VOL. 24 1943

The preceding discussion on the lower jaw deals with the conditions in gnathostome fishes. In the third part of this work (HOLMGREN, 1942) I have attempted to explain the jaw conditions in the light of those in Myxinoids (and Antiarchi) and Holocephalians. I refer to this work.

The problem of the upper jaw, the palato-quadrate (of the Selachians), is of incomparably greater interest and involves a great number of questions, of which the "pharyngo-mandibular" problem has been an object of numerous speculations and researches. In his paper on the morphology of the visceral apparatus of the Elasmobranches SEWERTZOFF (1923) has summarized the opinions concerning the morphology of the mandibular arch, pointing out that there were two principal opinions represented at that time. The opinion of GEGENBAUR, which the majority of authors have accepted, was that the present state of the mandibular arch in sharks is a primitive one, i.e. that the palato-quadrate includes the as yet undifferentiated pharyngeal and epal elements of the arch and that similarly the mechelian cartilage includes the undifferentiated ceratal and hypal elements. The other opinion, held by DEAN, SCHAUINSLAND, WOSKOBOINIKOW, claimed that the primitive mandibular arch was divided, like a branchial arch, into four elements: a pharyngeal, an epal, a ceratal and a hypal. Accepting this latter view, SEWERTZOFF quoted two possibilities for the behaviour of the pharyngo-mandibular: 1) the pharyngo-mandibular has completely disappeared or been reduced to an insignificant rudiment, or 2) it is fused on to some other skeletal element.

According to SEWERTZOFF (1923) the so-called orbital process of the palato-quadrate in sharks is the pharyngo-mandibular fused on to the epi-mandibular. In 1924 SEWERTZOFF changed his opinion in view of certain observations made by DISLER (SEWERTZOFF and DISLER, 1924) on *Somniosus, Mustelus, Scyllium* and *Squalus* (?), where there was found, at the upper end of the orbital process, a small cartilage or a prochondrial rudiment of a cartilage.[1] The element was considered to represent a pharyngo-mandibular. It was taken to be homologous with a small cartilage nodule described by IWANZOFF (1887) in *Pseudoscaphirhynchus Kaufmanni,* lying in close contact with the "processus palatobasalis lateralis" but far away from the palato-quadrate, with which, however, it is connected by a ligament. This small cartilage is embedded in the thick pad of tough connective tissue covering the palatobasal process in Acipenserids. In 1929 BUGAJEW studied this "pharyngo-mandibular" in different Acipenserids, where he found it developed very differently. In *Acipenser Gyldenstaedti* he found two cartilage nodules, in *A. stellatus* there was wide variation in the "pharyngo-

[1] In a specimen of *Somniosus* which I have investigated, no such cartilage is present, and in *Mustelus* and *Scyllium,* in which this cartilage is present, it does not join any orbital process, but constitutes this process. The cartilage of *Somniosus* must be accessory, as it may also be in *Squalus,* in which I have never found any trace of such a cartilage.

mandibular" elements. Here 4—6 such elements could form a series along the palotobasal ridge or the series could be fused to form an oblong cartilage. BUGAJEW has illustrated a frontal section through the palatobasal process of *A. ruthenus* (?). In the connective tissue outside it 4 cartilage nodules are seen. In adult specimens, however, only one cartilage is said to be present.

Very closely related with the "pharyngo-mandibular" problem is the question of the primary connection of the palato-quadrate with the neural skull i.e. the position of the dorsal end of the mandibular arch. GEGENBAUR (1872), referring to the conditions in Notidanids, claimed that this connection was situated in the otical region, where in these sharks the so-called *processus oticus* articulates against the postorbital process. SEWERTZOFF (1923) and other earlier and later authors were of the opinion that the connection is between the "orbital process" of the palato-quadrate and the "processus palatobasalis lateralis", as in other sharks and in Acipenserids. The palatine process anteriorly to the orbital represents, according to SEWERTZOFF, the upper end of the epimandibular. The orbital connection of the palato-quadrate is very old. SEWERTZOFF, however, says that he does not believe that this orbital articulation was a primary one. On the contrary, he believes that the primary connection of the pharyngo-mandibular was situated in the parachordal region. This primary connection, however, was not the same as the otical of the Notidanids. According to SEWERTZOFF, therefore, there may be three at least partly different connections to be considered.

In the primary mandibular arch HALLER (1924) recognized four portions: "Unterkieferstück, Zwischenstück, Oberkieferstück und Kieferaugenspaltstück". The "Zwischenstück" is said to be employed to form the articular tissue between the lower and the upper jaw. The "Unterkieferstück" is said to correspond to a hypo-branchiale, the "Oberkieferstück" to a ceratobranchiale and the "Kieferaugenspaltstück" to a pharyngo-branchiale. This "pharyngo-branchiale", however, takes no part in the formation of the future upper jaw but is said to disappear, and the "Oberkieferstück" alone develops into the palato-quadrate. The primary connection between the mandibular arch and the neurocranium is said to correspond fairly closely to the processus oticus of notidanids, but HALLER does not conclude that the "Kieferaugenspaltstück" corresponds to the processus oticus. He says: "In der Tat entspricht der Ort der Verbindungsstelle, die wir bei jungen Keimlingen sehen, auch ist hier die Abgangsstelle des zugehörigen Nerven des Trigeminus zu suchen, allein diese Verbindung kann trotzdem etwas Neues sein; in ihrer Beschaffenheit ist sie es sicher, denn sie steht in Abhängigkeit von der Ausbildung der Kiefer." The "Kieferleisten" develop through "Neubildung" ("Sprossung") from the "Oberkieferstück". According to HALLER, the upper ends of the "Oberkieferstücke" are said to be connected by a mesenchyma, the "Hypophysenpolster", in which the posterior part of the trabecula would

arise, whereas the anterior part of these elements, according to him, originate from the "Oberkieferfortsätze", i.e. the anterior parts of the palato-quadrate.

According to ALLIS (1923 and 1931), the polar cartilage (posterior part of the trabeculae (HALLER)) is the pharyngo-mandibular. The trabeculae and palatine process of the palato-quadrate (1931) are said to represent the dorsal and ventral halves of a premandibular arch. ALLIS' (1931) opinions are in part founded upon the findings of HALLER, in part purely theoretical.

A similar theory to that of ALLIS has been published by DE BEER (1931). His opinion that the trabeculae are visceral structures is based upon the following evidence: 1) The maxillary processes originate from the visceral mesenchyme associated with the mandibular arch. 2) The trabeculae are condensations in situ in the maxillary processes. 3) The trabeculae and the palatine processes of the palato-quadrate have a common origin. 4) There is no evidence af any proliferation from the premandibular somite. DE BEER's conclusion is that the trabeculae represent a premandibular arch. The polar cartilages might possibly be part of the mandibular arch.

VOL. 24 1943

From the preceding pages it seems evident that the problem of the morphology of the palato-quadrate and adjacent structures is far from solved and that the field is still open for new speculations and theories.

Objections are possible to the three last named theories quoted, some being so vital that the theories must be rejected as untenable.

In that part of this paper in which the early development of the trabecula is described (p. 12), it was pointed out that the trabecula arises in mesenchyme derived from the premandibular somite (and not as condensations in the maxillary processes), whereas the mesenchyme of the oral arch originates from the second division of the neural crest. In the area in which the two portions of mesenchyme meet is located the connection of the trabecula with the anterior end of the primary palato-quadrate, those elements, however, arising *in situ*. There could be no question of the one of these elements giving rise to the other. For these reasons I must reject the theory of the trabeculae representing part of any visceral arch.

At early developmental stages of *Squalus* I was able (HOLMGREN, 1940) to trace the origin of the polar cartilage. There it was proved beyond any doubt that the polar cartilage is derived from the mandibular somite. From the fact of its somitic origin it follows that this cartilage could not possibly be the pharyngeal element of the mandibular arch, which is of ecto-mesodermal origin.

The "Kieferaugenspaltstück" of HALLER, which, he says, represents the pharyngo-mandibulare but disappears, does not in fact disappear in Selachians but develops into the branchial ray apparatus of the spiracle and the lateral commissure, so that it could not be pharyngo-mandibular at all. (HOLMGREN, 1940).

The researches of SEWERTZOFF seem to provide evidence in support of the theory that the mandibular arch was once divided into parts homodynamous with those of a branchial arch. Supposing that the segmentation of the branchial arches as represented in selachians or ganoids is primary, then the mandibular and branchial arches of the ancestors of the selachians would once have been composed of homodynamous pieces. Thus far I am inclined to accept this view, but in my opinion the question is far more complicated than that.

In my description of the development of the selachian skull (HOLMGREN, 1940) three connections have been demonstrated between the rudiment of the palato-quadrate and the neural skull. One of these, the anterior, seems to be primary; the others develop later. The anterior connection (the ethmoid or orbital) is developed between the palato-quadrate and the anterior end of the rudiment of the trabecula. The next connection arises later than the ethmoid connection. It is between the polar cartilage and a posterior part of the palato-quadrate. This connection seems to be made up of the neural cranium and is thus a basitrabecular process. The hindmost connection, of which the earliest rudiment arises at the same time as the trabecula, corresponds to the "Kieferaugenspaltstück" of HALLER and is part of the spiracular complex. Part of it connects the posterior portion of the palato-quadrate with the postorbital process and is thus the "otic" process (processus oticus internus) (HOLMGREN, 1940). Like the otic process of the dipnoans it lies laterally to the hyomandibular nerve and the jugular vein. This process is not the same as the otic process of Notidanids (processus oticus externus). See p. 62.

In embryonic selachians the palato-quadrate is connected with the skull throughout its entire length, but this connection is not effected by cartilage but my membrane (HOLMGREN, 1940). The ethmoid connection in selachians ontogenetically breaks up to form an articulation between the palato-quadrate and the trabecular part of the skull. This articulation is that between the "orbital process" and the "processus palatobasalis lateralis" (SEWERTZOFF).

The "ethmoid" connection of the palato-quadrate in *Scyllium* at the earliest stage, when the palato-quadrate and the trabecula are present as determinable elements, lies at the anterior end of the trabecula. There the trabecula and palato-quadrate are continuous without any boundary line between them. On the trabecular side the inferior obliquus muscle of the eye tends to become attached to the connecting part. At a somewhat later stage the connecting bridge is about to differentiate into three parts. One of these by means of the eye-muscle insertion maintains its continuity with the trabecula, an anterior part is continuous with the rudiment of the orbitonasal lamina now forming, and the third joins the palato-quadrate later to form its orbital process. These three parts in *Scyllium* never separate fully as there will always remain

a ligamentous connection between them. The primary "ethmoid" connection always lies at the frontal end of the trabecula, where it has previously (HOLMGREN, 1940) been described as a lateral trabecular process, but it is to be clearly understood that this "process" must have at least a double prospective value. In *Scyllium* the connection always lies well in front, immediately behind the orbitonosal lamina, at the frontal end of the trabecula. The trabecula, however, grows longer, though most of the growth takes place behind its tip. In *Squalus* and *Etmopterus* the trabeculae also grow out, but in these genera the principal centre of growth lies anterior to the "lateral process" of the trabecula. The "ethmoidal" connection has therefore shifted backwards into the orbital region of the skull, and the future articulation has become an orbital. Thus different position of the articulation in squaloid and in galeoid sharks is not due to any "slipping" forwards of the orbital process along the trabecula in the latter.

VOL. 24 1943

The method of breaking up the ethmoidal connection is somewhat different in different selachians (HOLMGREN, 1940). In *Squalus* and *Etmopterus* the early conditions are the same as in the early *Scyllium* embryo. As the connection is severed, the main portion of the trabecular process forms a part—the orbital process—of the palato-quadrate and the rest the ligament joining that process with the trabecula. Thus the orbital process in *Squalus* and *Etmopterus* is never separate as it is in *Scyllium.* In *Raja* there is also an early continuity between the rudiment of the trabecula and that of the palato-quadrate. Through the breaking of this continuity no orbital process is formed at all, but the connection is extended to a ligamentous lamella, into the frontal end of which the obliquus inferior muscle is inserted. The posterior part of this lamella becomes extended caudad along the trabecula, thus forming an early connection between the entire frontal end of the palato-quadrate and the entire trabecula. The condition is similar in *Torpedo,* but there the obliquus inferior muscle retains its insertion in the trabecula (trabecular process), at the posterior end of the lamina orbitonasalis (nasal capsule). The connection between the trabecula and the palato-quadrate is thus formed by a membrane only. No orbital process is present at any stage of development.

The ligamentous membrane in sharks and rays that already at early stages connects up the trabecula with the anterior part of the palato-quadrate later extends to the otical region, incorporating the membranous part of the basi-trabecular process, if such a process is present, as is the case in the squaloid sharks (HOLMGREN, 1940). Posteriorly this membrane connects up with the spiracular complex. This membrane is especially interesting in *Scyllium,* as its medial part chondrifies along the trabecula. The chondrification is separate, forming the so-called subocular cartilage which later fuses on to the trabecula and the polar cartilage (HOLMGREN, 1940). To this subocular cartilage is added posteriorly the otical shelf, which is formed separately. By

this process of fusion is formed the subocular shelf that is so characteristic of galeoid sharks. The separate chondrification of the subocular cartilage seems to indicate a certain independence on the part of this cartilage in relation to the trabecula. Whether this independence is of primary importance or not, could not be decided from my material of *Scyllium*, but should it be so, the unchondrified membrane in other selachians would also be a structure independent of the trabecula. As the orbital process in *Scyllium* (and also in *Squalus* and *Etmopterus*) undoubtedly belongs to this membrane, one might be inclined to believe that the rest of the membrane would also be of palato-quadrate origin. There are some reasons of a histogenetical nature in support of this theoretical possibility. In *Squalus*, in which a great many developmental stages have been available for investigation, the process of separation between the trabecula and the palato-quadrate could be followed quite closely and the following observations could be made.

In 20 mm embryos of *Squalus* there is still no trace of chondrification in the rudiments of the trabecula and of the palato-quadrate. Nevertheless they are well differentiated from one another by the different size and arrangement of their cells. The cell nuclei of the trabecula are larger and more or less transversely or irregularly arranged. There is no denser core in the centre of the trabecula. The cells of the palato-quadrate are small and arranged concentrically around a core of more densely arranged cells of about the same size. The peripheral layers of the palato-quadrate cells on the medial side are in close contact with the trabecula. In the rudiment of the orbital process the cells are more densely arranged than in the rest of the palato-quadrate and there is no definite concentric arrangement. Towards the tip of the process the cells are larger and more embryonic, indicating a growth of the process.

At the 21 and 22 mm stages the trabeculae are becoming transformed into procartilage, whereas the palato-quadrate is still quite blastematic. The development of collagenous matter in the trabecula accounts for the different staining of the two parts in the Azocarmin-Mallory preparations. The trabecula stands out light blue, the palato-quadrate intensively red. In other respects these stages are the same as the preceding.

In the sections of the 22 mm stage the palato-quadrate is cut longitudinally (horizontally). There it is seen that those medial parts of the concentric cell-layers of the palato-quadrate which are in contact with the trabecula continue along the palato-quadrate also behind that point where the palato-quadrate and the trabecula have connection with one another. This fact supports the conclusion that the concentric layers in contact with the trabecula really belong to the palato-quadrate.

In an embryo of 22.5 mm body-length the trabecula is prochondrial, rather sharply outlined. The palato-quadrate is blastematic and the connecting part is the same as in the preceding stages, with one exception, viz. at the posterior

part where the palato-quadrate withdraws slightly from the trabecula. At this point, however, the connection is maintained, the periferal layers of the palato-quadrate cells being extended to form a connecting cell mass.

The 24.5 mm embryo (fig. 36) behaves in the same way as the preceding, but the trabecula is in process of chondrifying, the chondrification extending over the entire trabecula, which is well outlined. The processus orbitalis of the palato-quadrate has grown out considerably.

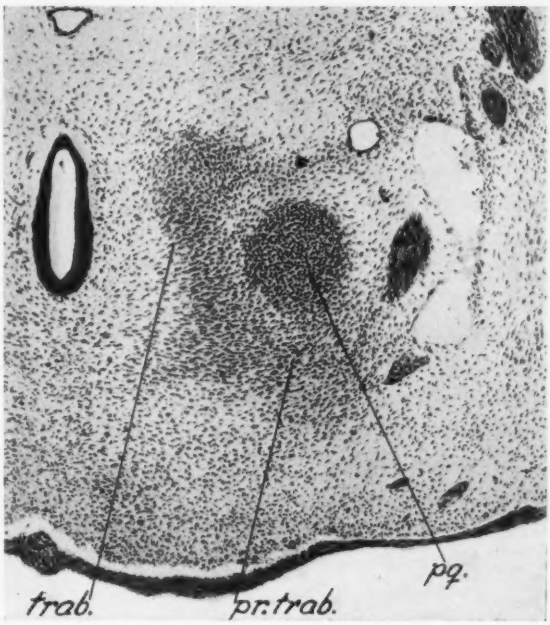


Fig. 36. *Squalus acanthias.* Embryo 24.5 mm. Transversal section through the anterior end of the palato-quadrate and the trabecle.

At the 25.5 mm stage the trabecula is uniformly weakly chondrified. In the core of the palato-quadrate the prochondrium is developing. The concentric layers of the anterior part of the palato-quadrate are, as before, in contact with the trabecula. As the trabecula and the palato-quadrate posteriorly withdraw from one another the connecting cell-layers are drawn out forming a short thick bridge between them. In this bridge the cells stand perpendicular to the transverse extension of the bridge, viz. preserve the position they had formerly as parts of the concentric layer of the palato-quadrate.

At the 26—27 mm stage this bridge has grown longer and thinner and is from now on with certainty determinable as the future membrane connecting the trabecula with the palato-quadrate.

The histological conditions during the development of the trabecula and the palato-quadrate thus seem to lend support to the theory of the palato-quadrate origin of the fascia joining the palato-quadrate with the trabecula. Applying this result to *Scyllium,* we must regard the subocular cartilage or shelf as a part of the palato-quadrate fused on to the trabecula.

The big ligament joining the "processus palatobasalis lateralis" with the processus orbitalis of the palato-quadrate develops at the tip of the orbital process. Accordingly it may also be a part of the palato-quadrate. In *Raja*

VOL. 24 1943

and *Torpedo* the corresponding ligament forms the foremost part of the lamella joining the palato-quadrate with the trabecula, thus including the orbital process, which, however, never becomes visible as a process.

Later on the trabeculo-palato-quadrate lamella extends caudad along the basis cranii and along the palato-quadrate. Its connection with the trabecula is strong, with the palato-quadrate rather weak. Caudally it includes the membranous basitrabecular process and connects up with an otical shelf portion and with the spiracular complex.

Such an otical shelf portion is developed, for instance, in *Scyllium,* in which there is a certain relation between this shelf and the spiracular complex. Inside this independently developed otical shelf portion passes the orbital artery (HOLMGREN, 1940). In *Heterodontus* a similar shelf is present, but its independent origin could not be established (for lack of suitable stages). in *Scyllium* as well as in *Heterodontus* a strong ligament (the lower postspiracular ligament of ALLIS, 1915) connects the posterior part of the shelf with the posterior inner part of the hyomandibula. In *Etmopterus* a shelf portion develops rather late in connection with the basiotic lamina (HOLMGREN, 1940). It lies, however, inside the orbital artery. In *Squalus,* according to DE BEER, the orbital artery at later stages of development becomes enclosed by the shelf.

The otical shelf cartilage will be considered at somewhat greater length in connection with the spiracular cartilage complex.

The "Kieferaugenspaltstück" of HALLER (1924) has proved to contain the rudiment of the spiracular complex. In embryos, in which the mandibular arch is just formed, the spiracular rudiment is found attached as an immediate dorsal continuation of the dorsolateral margin of a somewhat anterior part of the palato-quadrate portion of the arch (HOLMGREN, 1940). It forms a more or less triangular mesenchymatic lamella (otic process) anterior to the spiracular canal. In a dorsal direction it reaches up toward the frontal part of the auditory capsule. As the palato-quadrate grows frontally, the spiracular rudiment assumes a more caudal position. Simultaneously it extends dorsad and comes into contact with the postorbital process and fuses with it dorsally (HOLMGREN, 1940). Where the process passes the basal part of the auditory capsule it also connects with this part. Between the two connecting points the rudiment is bent laterad, embracing the hyomandibular nerve and the jugular vein, and forming a mesenchymatic "lateral commissure" (DE BEER) or otic process. Where the spiracular rudiment passes in front of the spiracle it is thickened, with densely arranged cells. This part later develops into the prespiracular cartilages, which may remain separate as in *Etmopterus,* or fuse into a more or less concave cartilage plate as in *Heterodontus, Raja* and *Urolophus.* Simultaneously with the development of the prespiracular cartilages their connection with the palato-quadrate becomes weaker or disappears altogether.

The general features of the development of the spiracular complex show that it corresponds to an otic process to which have originally been attached some branchial rays belonging to the pseudobranch. Later the series of rays separate from the process.

The reasons why I consider this otic process, and the branchial rays belonging to it, to be parts of the same complex are the following:

1) Both parts develop from a common mesenchyme connected with the palato-quadrate rudiment.

2) This mesenchyme has the position—in relation to the palato-quadrate —of a branchial ray bar, and as it gives rise to the branchial rays of the pseudobranch it represents at least a part of the branchial ray bar of the palato-quadrate.

3) On the lateral or posterior border of the processus oticus portion the spiracular rays are placed as in the hyoid arch in rays where the hyoid rays are attached to the pseudohyoid, which has originated through a fusion of the bases of the hyoid rays. The processus oticus might thus be considered as a "pseudo-palato-quadrate". In many selachians the processus oticus does not chondrify, though in most cases it chondrifies, forming the lateral commissure of DE BEER. In a 27 mm embryo of *Urolophus Halleri* the chondrification of the otic process (lateral commissure) is almost simultaneous with that of the prespiracular cartilage plate (formed by fused rays), and in this species the lower part of the otic process undoubtedly is a branchial ray belonging to the same series of rays as those forming the prespiracular plate or cartilage (fig. 37). In *Urolophus* it thus seems clear that the otic process is formed by at least one ray.

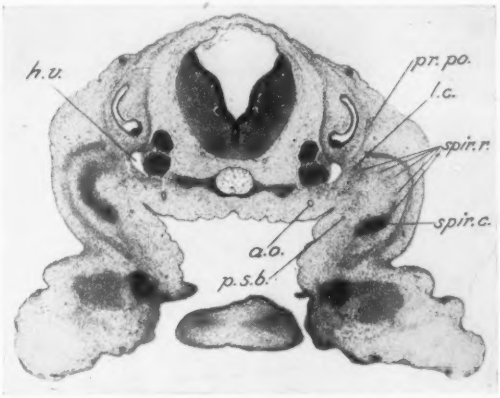


Fig. 37. *Urolophus Halleri*. Embryo 27 mm. Transverse section of the head level with the prespiracular cartilages and the lateral commissure.

It seems somewhat doubtful whether the otic shelf rudiment belongs to the spiracular system. In *Scyllium* its connection with the spiracular system might be evidenced by the insertion in its posterior part of the lower postspiracular ligament as described by ALLIS (1915), the corresponding ligament being inserted in *Urolophus* into the posterior lower part of the lateral commissure (otic process). In *Heterodontus* the otic shelf has the same relation to the orbital artery as in *Scyllium,* but the posterior end of the ligament is attached to the upper end of the hyomandibula instead of its lower posterior half.

VOL. 24 1943

Finally in *Etmopterus* the artery and the ligament fail to offer any argument in either direction.

The "otic process" which has been discussed in the preceding pages is not the same as that defined in 1876 by HUXLEY and typically occuring only in Notidani among living selachians. The processus oticus of *Heptanchus* articulates against the postorbital process. The constrictor dorsalis muscle is inserted in its inside, the adductor mandibulae in its outside, as has been demonstrated, for instance, by EDGEWORTH. The processus oticus of *Ceratodus* lies inside the constrictor dorsalis and so does the processus oticus described by me. From this very statement the homology between the two processi otici may appear somewhat doubtful. This statement supported by the following evidence will make it certain that the processus oticus of *Heptanchus* is something quite different from that of my description and that of the *Dipnoi, Amphibians* etc.

In *Etmopterus* the palato-quadrate has a big processus oticus, but it does not as in *Heptanchus* reach the postorbital process. It is proved to be the same processus oticus as that of *Heptanchus* by the insertion into it of the muscles: the constrictor dorsalis on its inside and the adductor mandibulae on its outside. The development of the *Heptanchus* process could not be studied in detail owing to the lack of suitable embryonical stages, but in *Etmopterus* it was possible to follow its development. To establish the non-homology of the two processi otici (of *Heptanchus* and of my description) it is of course sufficient to point out that they occur simultaneously in *Etmopterus* (and other selachians), but the development of the "*Heptanchus* process" is so interesting that it must be summarized here. In my account of the different stages of *Etmopterus* (HOLMGREN, 1940) is described the "extra-palato-quadrate blastema". This blastema in *Etmopterus* and also in *Heptanchus* develops early just below the skin laterally to the palato-quadrate. It sinks down towards it and connects up with it. The constrictor dorsalis muscle makes an insertion in the dorsal side of the connecting part, the adductor mandibulae muscle in its ventral side. Subsequently the extrapalato-quadrate blastema shifts to the dorsal edge of the palato-quadrate and fuses completely with it, so that the insertion of the constrictor dorsalis comes inside it and the adductor mandibulae outside it. The "extra-palato-quadrate blastema" forms the "otic process". The development of the other "otic process" (lateral commissure) has proceeded simultaneously.

For the two different otic processes differentiating names must be introduced and I propose to call that of *Heptanchus* and the other Notidani the *processus oticus externus* and that of probably all other vertebrates the *processus oticus internus*. Even in *Heptanchus* both processes are present, the internus, however, reduced to a thin indistinct connective tissue membrane in which is embedded the dorsal pocket of the spiracular canal, as the case

normally is in Selachians and also in *Dipnoi*, in which there is a certain relation between the corresponding pocket, the organ of PINCUS, and the otic process.

More or less related to the system of the spiracular cartilages are some raylike structures attached to the most posterior end of the palato-quadrate. The structures especially developed in *Torpedo* and *Urolophus* but also present in *Etmopterus* and *Raja*, and perhaps also in *Scyllium*, will be discussed together with the hyomandibular problem.

In the preceding discussion of the palato-quadrate in Selachians an essential part has hitherto been neglected, viz. the so-called palatine portion. In the first part of this work (HOLMGREN, 1940) the development of this portion of the palato-quadrate has been followed in the different selachian species, and in all cases it has been shown that it develops into a blastema common to it and to the teeth plate. This development commences later than that of the rest of the palato-quadrate. Therefore the palatine part always has a certain independence in relation to the rest. This independence becomes accentuated in *Etmopterus* as it is retained after the chondrification has begun, so that a relatively independent cartilage forms the palatine part of the palato-quadrate at a comparatively advanced stage of development. Quite early in this development the teeth blastema has become separated from the future palatine cartilage. Later the teeth become attached to it, but at that time the palatine cartilage has fused on to the rest of the palato-quadrate.

In the preceding pages those structures belonging to the palato-quadrate system which seem to be of some importance for a palato-quadrate theory have been mentioned. The many facts thus considered seem to complicate the problem somewhat, so much indeed that a theory covering all facts is difficult to find.

In order to advance a branchial-arch theory in respect of the mandibular arch, based upon the conditions in sharks and rays, we must take into account the following structures.

1) the palato-quadrate proper and its connections with the neurocranium,
2) the orbital process,
3) the palatine process,
4) the lateral commissure (processus oticus internus),
5) the spiracular cartilages (prespiracular, postspiracular, lateral) and the the otical shelf cartilage,
6) the processus oticus externus (extra-palato-quadrate),
7) the meckelian cartilage, posterior centre,
8) the meckelian cartilage, anterior centre,
9) the symphysial cartilage of the mandible,
(10) the palato-quadrate symphysial cartilage).

VOL. 24 1943

Of all these parts the last named does not belong to the mandibular arch as it develops as a part of the medial ventral field of the preoral part of the head and not from the oral arch (HOLMGREN, 1940).

SEWERTZOFF's (1923) interpretation of the mandibular arch, according to which it consists of a pharyngomandibular (orbital process), an epimandibular (palato-quadrate proper with palatine part), a ceratomandibular (meckelian cartilage) and a hypomandibular part (hypothetical), seems to be a sound basis for the further development of the question. SEWERTZOFF did not, however, take into consideration the fact that the general idea of a branchial arch includes two different pharyngobranchials, viz. a supra- and an infra-pharyngobranchial; moreover, he did not consider the branchial bar and its possibilities. Consequently his interpretations became incomplete. SEWERTZOFF considered the palatine part of the palato-quadrate to be merely a frontal continuation of the palato-quadrate proper, whereas it is a separately formed constitutent of the mandibular arch. In my opinion the palatine part represents the infra- pharyngomandibular and the orbital process the supra-pharyngomandibular. In squaloid sharks the orbital process is formed in continuum with the palato-quadrate proper as does the supra-pharyngobranchial in *Amia* and *Lepidosteus,* in which the infra-pharyngobranchial has a separate origin. In *Scyllium* the orbital process has a separate origin as the supra-pharyngobranchial in *Acipenser* (in which the infra-pharyngobranchial develops as a process of the epibranchial).

If the orbital and the palatine processes are the pharyngomandibulars, it follows that the palato-quadrate proper (the palato-quadrate without these processes) is the epimandibular.

The ventral part of the mandibular arch consists of the two centres of the meckelian cartilage (VAN VIJHE, 1922, HOLMGREN, 1940), which apparently correspond to the ceratobranchial and the hypobranchial respectively of the branchial arch. They are consequently considered to be the cerato- and hypomandibular. In or closely behind the symphysis mandibulae lies an unpaired cartilage nodule, which may perhaps be a mandibular copula, comparable with the copulae of the branchial arches and the postmandibular plate in the Antiarchi (HOLMGREN, 1942).

Behind each branchial arch lies a branchial ray bar, in which the branchial rays develop. In many sharks basal parts of the rays, especially those of the hyoid ray bar, are fused, forming cartilaginous lamellas from which the rays extend. In the Batoids the hyoid ray bar through the fusion of basal parts of rays gives rise to the "pseudohyoid" with its rays. This pseudohyoid with rays exemplifies a ray bar that has become emancipated from its arch, forming a relatively independent complexity. Similar is the behavior of the mandibular ray bar. There a part of the bar is consumed by the formation of the lateral commissure or processus oticus internus, while the rest forms the mandibular

(spiracular) rays. A number of these rays form the pre-spiracular cartilage or cartilages. One ray is the postspiracular cartilage, which becomes more or less intimately fused on to the hyomandibula or forms a sort of embryonic symplectic between the hyomandibular arch and the mandibular (in *Potamotrygon* and *Mobula* there are two rays, connecting the two arches). In *Raja* a laterad directed raylike structure may also possibly be a mandibular ray.

As to the processus oticus externus or extra-palato-quadrate I must confess that I am at a loss to explain it fully. In the branchial arch system there is, as far as I know, no part present which could foreshadow the external otic process. Therefore this process must be regarded—at least provisionally—as a special acquisition of the palato-quadrate. As an external otic process is present in the oldest shark types (*Cladoselache, Protacrodus* etc.) and in Acanthodians, it is very probably an early acquisition. It is characteristic of the external otic process, when perfectly developed, that it attains the postorbital process and articulates with it. This development of the process is probably not primary, as some older Acanthodians (*Climatius, Mesacanthus*) had no such articulation, and among the sharks the process is very differently developed, totally lacking in *Scyllium* (in embryos and in adults) and adult rays (but present in embryos). The devonian *Cladodus* had no postorbital articulation.

In Elasmobranch fishes no explanation of the extra-palato-quadrate could be obtained, but turning our attention to the Crosopterygians we there meet with the so-called suprapterygoid series of substitution bones in *Megalichthys* extending along the upper border of the deep part of the palato-quadrate and also present(?) in *Eusthenopteron*. This series gives the impression of a series of broad and flattened rays added to the upper border of the palato-quadrate proper. The extra-palato-quadrate in sharks and rays seems to correspond closely to such a bone and could therefore be looked upon as a portion of the palato-quadrate ray bar of which the posterior portion gives rise to the spiracular complexity. The ascending process or epipterygoid may also belong to the same category as the suprapterygoids and the extra-palato-quadrate.

In fig. 38 my interpretation of the mandibular and hyoid arches of a generalised selachian fish is diagrammatically represented.

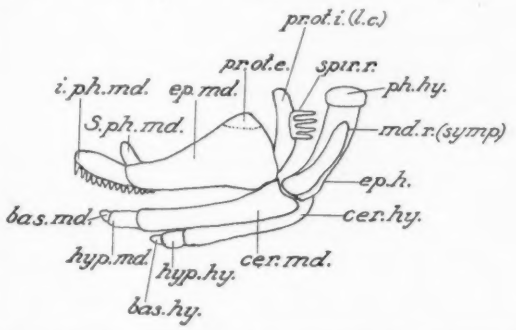


Fig. 38. Diagrammatical representation of the general morphology of the selachian mandibular and hyoid arches.

The "postpalatinum mediale" (BUGAJEW, 1930).

In *Scymnus* GEGENBAUR described an anterior medial unpaired cartilage of considerable dimensions, which he considered to be a separate part of the palatine process. BUGAJEW (1930) described and figured this cartilage, and named it the "postpalatinum mediale". He compared it with the complicated palatine apparatus in Acipenserids, a comparison that is undoubtedly of great interest. But BUGAJEW did not give any explanation of the morphological value of it either in *Scymnus* or in Acipenserids. I have no embryological material of *Scymnus,* so that I have been unable to study the development of this remarkable cartilage, but from a young specimen I found that veins belonging to the longitudinal venous plexus of the soft connective tissue in the roof of the bucal cavity enter the cartilage and are embedded in it. Seeing that in the other parts of the mandibular arch bloodvessels never seem to enter any cartilage, it may be possible to conclude from this fact that the unpaired cartilage is of a more special nature than the canonical mandibular arch cartilages. This conclusion is supported by an investigation into the palato-quadrate symphysis in an *Etmopterus*-embryo of 43 mm length. Between the two branches of the palato-quadrate lies a disc of blastematic tissue. This symphysial tissue, as is learned from earlier stages, belongs to that part of the medial area of the premandibular portion of the head which in all selachians investigated enters between the two palatine processes (HOLMGREN, 1940). At the 43 mm stage this tissue posteriorly broadens to a blastematic plate lying at the upper and posterior border of the symphysis. Laterally this plate is prochondrial. Vein-branches belonging to the longitudinal palatine vein plexus are embedded in it. It is obvious from this fact that the "postpalatinum mediale" is ontogenetically present in *Etmopterus,* although in a rudimentary state. In *Etmopterus* it is characterized as a symphysial structure, morphologically not belonging to the mandibular arch, and the same is no doubt the case in *Scymnus.* No such cartilage has been found in other selachians. In Acipenserids the postpalatinum complex differs from that of Selachians as no blood vessels enter the cartilages. Veins are, however, present surrounding the separate cartilages of the complex. From the general arangement and blood supply the conclusion might be drawn that the postpalatinum mediale in *Scymnus* and in Acipenserids may be homologous. Unfortunately, however, the decisive piece of evidence has been missing, viz, the origin of the symphysial tissue in Acipenserids was still unknown. Supposing the symphysial tissue has a different origin in the two groups of fishes, we should scarcely be justified in putting structures, originating from different sources, together under a common name. It was necessary therefore to investigate *Acipenser* in order to ascertain the development of its palato-quadrate symphysis. An 8 mm embryo of *Acipenser stellatus* gave the answer to the

question. In this embryo it is clearly seen that a medial area from in front enters between the anterior tips of the palato-quadrates forming the symphysis into which the postpalatine cartilages subsequently differentiate. From this observation it seems probable that the postpalatinum mediale in Selachians and Acipenserids is homologous.

The morphology of the hyomandibula in sharks and rays and its relation to the auditory capsule and to the mandibular arch.

In his work "The Homologies of the Hyomandibula of the Gnathostome Fishes" (1915) ALLIS has reviewed the earlier literature about the hyomandibulare problem in sharks and rays. It is not necessary therefore to repeat more than the essentials.

Sharks. There seem to have been two different opinions about the hyomandibula in sharks. According to GEGENBAUR (1872), PARKER (1876), DOHRN (partly) (1884—85), SCHAUINSLAND (1903) and others, the hyomandibula represents the upper half of the hyoid arch: the epi-pharyngohyal. According to LUTHER (1909) the hyomandibula is the epal element, the pharyngeal being rudimentary or missing. ALLIS (1915) has joined this opinion. HOLMGREN and STENSIÖ (1936), as a preliminary result of the present investigation (HOLMGREN, 1940), have accepted the pharyngo-epihyal theory.

Rays. According to GEGENBAUR the hyomandibula of the rays is homologous with that of sharks. The interpretation of the hyomandibula in rays given by PARKER, DOHRN and ALLIS is due more or less to a misinterpretation of the branchial ray bar as belonging to the inner hyoid bar system. KRIVETSKY (1917) has stated that the inner bar is reduced to the hyomandibula, whereas the rest of the "arch" is formed by fused parts of branchial rays. He calls this part the "pseudohyoid". ALLIS says that the hyomandibula is undoubtedly the pharyngeal element of the hyoid arch. According to HOLMGREN and STENSIÖ (1936), basing their theory upon a preliminary result of the present investigation, the hyomandibula of the rays is believed to correspond primary to the epal element.

The hyomandibula articulates on the outside of the auditory capsule. The differences in the position and configuration of the articular facet present in sharks and rays have to some degree been studied by, for instance, GEGENBAUR and HOFFMAN. The latter (1913) states that the articular surface in rays is situated upon a horizontal cartilage process at the ventro-lateral border of the auditory capsule, called by him the "Rochenvorsprung". The articular facet in sharks is more transversely located, lies more dorsally and is characterized by a posterior, more dorsal process: a "Haifortsatz". The articulation in rays is often divided into two facets. KÄLLBERG states that in

VOL. 24 1943

advanced stages of development the difference is not very distinct. According to his notes, he did not find any real difference between the articulation in rays and sharks. He did not, however, investigate this question at early stages of development. Such an investigation yields other and very interesting results.

From the previously given descriptions of the earlier developmental stages of *Torpedo* and *Raja* (HOLMGREN, 1940) it is obvious that between the auditory capsule and the future hyomandibula there is a pad of blastematic cells covering the ventrolateral side of the auditory capsule about from the hyomandibular nerve to the posterior corner of the capsule. According to the description there given this pad is at early stages separated from the auditory capsule blastema but is confluent with the blastema of the hyomandibula. The mesenchymatic cell mass is thereby proved to be visceral of origin, belonging to the hyoid arch. Later the mesenchymatic mass fuses on to the auditori capsule and partly at least separates the latter from the hyomandibula.

Anteriorly to the future hyomandibular articular facet this cell mass gradually forms a shelf portion posteriorly to the hyomandibular nerve. That portion of the cell mass which is situated between the articular part of the hyomandibula and the auditory capsule is used to form partly the border ridges of the articular facet, partly the intra-articular connective tissue and later the linings of the articular fossa. That part of the mesenchymatic pad lying posteriorly to the hyomandibula in *Torpedo* fuses with the basiotic lamina and forms the occipital shelf upon which the glossopharyngeal ganglion rests. In *Raja* it behaves as in *Torpedo,* but posteriorly extends dorsad to embrace the glossopharyngeal nerve and the jugular vein on their outside and fuse with the lateral border of the occipital surface of the skull dorsally to the nerve and vein. From the posterior part of the shelf thus formed a strong (interarcual) ligament issues, connecting the shelf with the upper end of the epibranchial of the first branchial arch. (During a 47 mm stage of *Raja* this ligament shifts to the upper caudal corner of the hyomandibula.) In *Urolophus* similar conditions are apparently present, but our youngest stage is too advanced to permit of an investigation of the development from the very beginning. The 27 mm stage, however, is sufficiently young to show that the tissue pad is present as in *Torpedo* and that the said ligament also occurs. In *Torpedo* a dorsal lamella extending from the anterior part of the pad enters between the otic capsule and the jugular vein. This lamella later fuses with the otic capsule. Part of the hyomandibular levator muscle originates in this lamella. In the rays there is thus present a visceral element, which fuses with the auditory capsule. This element belongs to the hyoid arch. The interarcual ligament is also present in *Torpedo.*

In the sharks that have been investigated (HOLMGREN, 1940) a tissue mass corresponding to that in rays is also present. But here the future of this cell

mass is different. Anteriorly to the hyomandibula the cell mass fuses with the skull behind the hyomandibular nerve, there forming o short ridge, which extends below the anterior part of the hyomandibula forming the anterior part of the border ridge of the articular facet. The rest of the mesenchymatic cell mass does not fuse with the auditory capsule but remains with the upper end of the hyomandibula, where, especially in *Scyllium*, it is distinctly seen forming the upper end of this element. It is well separated from the auditory capsule and posteriorly it is connected by a strong ligament with the upper end of the epibranchial of the first branchial arch. Subsequently the said mesenchymatic rudiment fuses on to the hyomandibula and chondrifies together with it.

These conditions in sharks compared with those in rays give the key to our understanding of this peculiar mesenchymatic rudiment as the pharyngeal element of the hyoid arch, which in rays almost completely fuses with the skull, in sharks for the most part with the hyomandibula. Thus the articular conditions in sharks and rays are quite different owing to the different behaviour of the pharyngohyal, since in sharks the articulation is situated between the pharyngohyal and the skull, in rays between the pharyngohyal and the epihyal. The distinction between "Haifortsats" and "Rochenvorsprung" has acquired a deeper meaning than it had before.

VOL. 24 1943

The preceding statements, based upon the investigations into the different embryonic stages given in the first part of this work (1940) have demonstrated that the upper end of the "hyomandibula" is differently composed in sharks and rays. This interpretation only agrees with that of previous authors in the assertion that the "hyomandibulars" of sharks and rays are different. Before it was proved by Russian anatomists (KRIVETSKY a.o.) that those parts which since GEGENBAUR had been considered to be the epal and ceretal elements of the hyoid arch in rays, are in reality the bases of rays of the hyoid arch, fused on to one another to form a "pseudohyoid", a correct comparison was hardly possible between the hyomandibula of sharks and rays. In the history of the hyomandibular homologies the work of ALLIS stands out as a very brilliant effort to arrive at an understanding of the selachian hyomandibula. ALLIS used the hyomandibular ligaments as landmarks for the comparison between sharks and rays. In *Scyllium* there are two postspiracular ligaments, a superior and an inferior (RIDEWOOD, 1896). The superior has "its attachement on the auditory capsule dorsal to the vena jugularis". Ventrally it is attached "partly on the palato-quadrate and partly on the outer surface of the lower end of the hyomandibula. The nervus hyomandibularis facialis runs outward between the ligament and the hyomandibula, posteriorly to the one and anteriorly to the other." To the characteristic description of this ligament may be added the following. In embryos of *Scyllium* (40 mm and younger) it is clearly seen that the upper part of this ligament is formed at the posterior

upper part of the (ligamentous) subotical shelf. From there it extends in the direction (as described by ALLIS) towards the lower end of the outside of the hyomandibula, where it becomes inserted immediately anteriorly and ventrally to the levator muscle of the hyomandibula. Before insertion it gives off a branch to the palato-quadrate, as described by RIDEWOOD (1896). Another somewhat thin branch was followed caudad and ventrad. It is inserted into a short ligament joining the upper end of the ceratohyal with the rear part of the lower jaw. That part of the upper postspiracular ligament which is inserted into the hyoid arch may possibly correspond to the hyosuspensorial ligament in Dipnoi. The inferior ligament is attached at the base of the skull below and behind the trigeminal foramen. The other end is fixed to the postero-internal edge of the lower half of the hyomandibula and partly to the upper end of the ceratohyal. According to ALLIS, the inferior postspiracular ligament corresponds to a musculus interarcualis dorsalis II, as described by VETTER, which in the branchial arches connects the epibranchial and pharyngobranchial of an arch. In the hyoid arch it has been reduced to a ligament, and as the pharyngeal element of this arch disappeared it has found a new place of insertion on the basis of the skull. I think the homologization may be satisfactory, but another explanation seems possible. This other explanation is as follows: The infrapharyngobranchials are transformed into a blastema at the anterior border of the epibranchials close to the lining epithelium. In transverse sections the rudiment is found ventrally to the dorsal branchial aorta. In the hyoid arch of ganoids a process of the hyomandibular blastema is found in the same position as the infrapharyngobranchial rudiments. This process no doubt corresponds to the infrapharyngeal element of the branchial arches. In *Scyllium* at the antero-dorsal part of the hyomandibular blastema a special blastema or blastematic process is found lying ventrally to the dorsal aorta. In *Scyllium* this blastema subsequently abuts on to the neurocranium, becomes ligamentous and, connecting up with the subotical shelf, forms the lower postspiracular ligament. The lower postspiracular ligament thus corresponds in my opinion to the infrapharyngobranchial process of the branchial arches. It therefore is an infrapharyngohyal, never chondrifying in living sharks or rays. In all selachians a ligament connects the anterior border and the inside of the hyomandibula (epihyal) with the basis cranii. This ligament is not the lower postspiracular ligament. Both ligaments are present in *Scyllium* and *Heterodontus,* for instance, a lower postspiracular ligament is present, but there it connects the otical shelf not only with the dorsal end of the ceratohyal but also with a lower posterior inside part of the hyomandibula. In no other shark or ray have I found a lower postspiracular ligament. In *Chimaera,* where a real infrapharyngohyal (as well as a suprapharyngohyal) is present, no such ligament could be located. There the hyomandibula is connected with the posterior part of the palato-

quadrate by a very strong (hyosuspensorial) ligament. *Chimaera* is the only elasmobranch fish where the hyal arch is complete. It might perhaps therefore be assumed that the typical relation between the efferent branchial artery and the pharyngeal elements would also exist in the hyoid arch. Such, however, is not the case, as the efferent hyoid artery passes behind both pharyngeal elements instead of between them. If this is the case in Holocephalians, it is not surprising that in sharks and rays the position of the efferent hyoid artery could not be used as an argument to prove that the lower postspiracular ligament represents the infrapharyngohyal.

ALLIS found this ligament to exist in sharks but not in rays. According to my experience it is present also in rays, but here the ligament is quite short and not very well differentiated from the articular capsule of the hyomandibula. ALLIS also believed in the general existence of the superior postspiracular ligament in sharks. I have established its existence in *Scyllium* and *Heterodontus,* but failed to find it in *Squalus* and *Etmopterus.* Nor could I find the ligament in any ray, at least not in a state sufficiently typical to allow of an identification. Moreover, ALLIS found no typical ligament in *Raja* and *Torpedo,* but connective tissue membranes and bundles were present, which might be the representatives of the superior ligament. In my series I have also found the structures described by ALLIS, but I could not be convinced of their being homologous with the well-defined ligaments in *Scyllium* and *Heterodontus.* That "ligamentous tissue in *Raja* which is firmly and directly attached ventrolaterally to the anterior portion of the external surface of the hyomandibula" might, of course, be homologous with the ligament connecting the hook-like process of the hyomandibula in *Torpedo* with the auditory capsule, but as the hook-like process in *Torpedo* is shown (HOLMGREN, 1940) to be a part of the spiracular cartilage system, the homology seems to be doubtful. In fine, these ligaments provided no clear evidence of the non-homology of the hyomandibula of sharks and rays. Proof of this non-homology is, however, afforded, as explained earlier, by the different primary anterior attachement of the interarcual ligament between the hyomandibular arch (pharyngohyal) and the epibranchial of the first branchial arch.

Independent "pharyngohyals" have been described only from *Stegostoma, Mustelus* and *Galeus,* where LUTHER (1909) found 2 such cartilages in *Stegostoma* and one in the others. In all three genera the "pharyngohyals" were found to be connected with the hyomandibula. As to the two cartilages in *Stegostoma* I cannot express any definite opinion, as LUTHER's paper contains no details concerning their relation to the interarcual ligament. In *Mustelus* and *Galeus* the small "pharyngohyals" apparently lie in this ligament, as they do in *Urolophus,* in which we have found, in older embryos, two such small cartilages. In *Urolophus* the two cartilages are embedded in the ligament,

VOL. 24 1943

which in this genus is attached anteriorly to that part of the skull which is formed by the true pharyngohyal. In the case of *Urolophus* it could hardly be a question of pharyngohyal elements but of cartilages forming in the ligament i.e. interarcual cartilages. In rays it is a well known fact that interarcual ligaments chondrify, and I would suggest that all these "pharyngohyals" represent the most anterior set of interarcual cartilages. In the sharks they are associated with the hyomandibula in that the upper end of this cartilage represents the true pharyngohyal. I conclude therefore that in adult sharks and rays the existence of separate pharyngohyals has not been demonstrated and probably never will be.

The lower end of the hyomandibula also exhibits some features of considerable interest from a morphological point of view. Considering that the gill-cleft between the mandibular and the hyomandibular arches was at an early phylogenetical stage[1] a normal gill-cleft, which later closed its lower parts, it must be obvious that the supporting functions of the hyomandibula in relation to the mandibular arch arose in connection with the rudimentation of this gill-cleft (ALLIS, 1925, HOLMGREN, 1942). How could this supporting function theoretically have originated? During a phylogenetical stage when the cleft was a normal gill-slit, there was no connection between the tissues of the mandibular and the hyal arches. The mandibular arch as well as the other visceral arches probably had its rays, as is evidenced by the spiracular cartilages present in modern selachians. As phylogenetically the gill-slit got its ventral part closed as far as to the mandibular joint, the rays belonging to the mandibula excepting perhaps the hindmost disappeared[2], whereas some rays on the palato-quadrate were retained. The closing of the gill-slit entails the fusion of the mesenchymatic tissues of the oral and hyoid arches and probably also the near approach of these arches to one another. As the closure of the branchial cleft proceeded dorsad, the most ventral of the retained rays of the mandibular arch lost their function as branchial rays and would therefore have disappeared if they had not been usable for a new purpose. At this stage of the closure of the gill-cleft the lower end of the hyomandibula and the posterior end of the palato-quadrate and of the mandibula were brought nearer to one another within a common mesenchyme. The lowest branchial ray of the quadrate or the highest of the mandibula[3]

[1] This stage must have been very early, probably pre-gnathostomian, as a spiracle was already present in *Antiarchi* and Arthrodires (STENSIÖ). In selachian embryos, where the first branchial opening is slit-like it does not extend to behind the lower yaw (ceratomandibular), and is thus no perfect gill-slit. Nevertheless we must presume that there was once a perfect gill-slit behind the mandibular arch.

[2] They lost their function as branchial rays and acquired no other function that could have preserved their existence.

[3] It is not possible to tell whether this ray is a palato-quadrate or a mandibular ray, as it forms at the point of the mandibular arch where the articulation will be established later. In the adult the articulation is mostly between the hyomandibula and the mandible and therefore the connecting ray may perhaps be the hindmost mandibular ray.

could now come into contact with the hyomandibula and perhaps also connect with it, thereby consituting an organic connection between the mandibular arch and the hyomandibula. As this ray (probably) articulated with the palato-quadrate or with the mandible, there would then arise by means thereof an articulation between the mandibular arch and the hyomandibula. (Compare below).

What is regarded above as a theoretical possibility in reality corresponds to what ontogenetically seems to have taken place in *Urolophus*. In the descriptions of the developmental stages in *Urolophus* (HOLMGREN, 1940) it was demonstrated that a prochondrial rod, inserted into the mandibular arch immediately behind the spiracular cartilage, fuses on to the external surface of the lower end of the hyomandibula. The hyomandibular levator muscle, originally attached to the rod just mentioned, invades the lateral surface of the hyomandibula and in part firmly inserts itself into it. This rod must be a ray belonging to the mandibular arch and corresponding to a medium ray of a typical branchial arch (HOLMGREN, 1940). The arguments on which these conclusions are based are as follows: In a branchial arch the constrictor dorsalis and ventralis muscles anteriorly to each gill-slit are inserted into the medium ray of its arch and are innervated by the spinal nerve belonging to the arch. The afferent branchial artery passes ventrally to the medium ray. In the hyoid arch the conditions are the same, in spite of the fact that in conjunction with the formation of a pseudohyoid in rays the rays of this arch have been carried caudad. In the mandibular arch the constrictor dorsalis, of which the principal mass lies anteriorly to the spiracle laterally covering the prespiracular cartilages, sends down a portion against the posterior surface of the palato-quadrate. The muscle here becomes ligamentous and finally attaches itself to the free anterior part of the rod, the medium part of which is fused on to the hyomandibula. The afferent pseudobranchial artery (= afferent artery of the mandibular arch) passes below the rod on its way to the pseudobranch.

The conclusion to be drawn from these facts could scarcely be another than that the above-mentioned rod is a (branchial) ray belonging to the mandibular arch and probably the medium ray of that arch.

If, then, in *Urolophus* a mandibular ray takes part in the formation of the hyomandibula it is questionable whether that condition is present in *Urolophus* only or whether it is a general occurence in sharks and rays and if the latter alternative is true, how it has developed in the different species here investigated.

Let us begin with *Torpedo*. In *Torpedo* the posterior part of the "branchial ray bar" of the palato-quadrate—the rudiment of the postspiracular cartilage —early develops into a blastematic process rising immediately in front of the hyomandibular rudiment and partly in contact therewith. This blastema lies

VOL. 24 1943

posteriorly to the spiracular canal. As it becomes prochondrial it is clearly seen that it is ventrally connected with the ventral part of the rudiment of the prespiracular cartilage and also with the posterior part of the palato-quadrate. It must therefore be considered to be of the same nature as the prespiracular cartilage, viz. a branchial ray (or ray-bar portion) of the mandibular arch. On the other hand, this postspiracular cartilage is connected with approximately the medium portion of the anterior border of the hyomandibula. In their further development the pre- and postspiracular cartilages form an unity whose connection with the palato-quadrate is weakening, whereas the connection with the hyomandibula becomes strengthened. By this process the postspiracular cartilage becomes transformed into the well-known hooklike process of the hyomandibula, which thus represents a mandibular (branchial) ray fused on to the hyomandibula. Dorsally to this process runs the hyomandibular nerve, and in its base and immediately ventrally to it the spiracular portion of the constrictor muscle of the mandibular arch is inserted into the hyomandibula.

In *Raja clavata* the conditions are in principle similar to those in *Urolophus*, the mandibular ray, however, being smaller and lying between the posterior end of the palato-quadrate and the anterior ventral corner of the hyomandibula, with which it fuses rather early. The tendon rudiment of the constrictor muscle in the early embryo is connected with the ray rudiment but later shifts dorsad to the anterior edge of the hyomandibula. In *Raja* there is added to this rudiment another, lying on the lateral surface of the hyomandibula (HOLMGREN, 1940). Into this rudiment is inserted a part of the levator muscle. I consider this rudiment to be a detached portion of the same mandibular ray, fusing on to the lateral surface of the hyomandibula and corresponding to the distal part of the ray in *Urolophus*. In *Potamotrygon* two mandibular rays connect the posterior end of the mandible with the lower end of the hyomandibula without fusion with either cartilage but forming articulations with the same.

The preceding discussion of mandibular ray elements has demonstrated that such elements are present in all investigated Batoids. Now the question arises: Are they perhaps a characteristic of other selachian fishes? The answer to this question is given us by a 30 mm stage of *Etmopterus*. The following lines are quoted from the description of this stage (HOLMGREN, 1940): "The frontal part of the hyomandibula is covered by an oblong blastema ventrally connected with the mandibular arch at about the point where the lower jaw articulation will become established." Some additions will now be made to this summary description. 1) There is a connection between this blastema—the postspiracular—and the blastema of the presspiracular cartilages lying in front of it. 2) The spiracular portion of the constrictor muscle at the 30 mm stage becomes inserted into the blastema of the posterior

end of the palato-quadrate into which is inserted the postspiracular blastema. Later the insertion chiefly shifts to the posterior end of the palato-quadrate. 3) The postspiracular blastema lies ventrally to the hyomandibular nerve, and 4) part of the levator muscle of the hyomandibula is inserted into it. None of these statements is inconsistent with the conclusion that this postspiracular blastema is homologous with that of *Urolophus* and of *Torpedo*. A simple comparison between the figs. 156, 171 and 81 in HOLMGREN (1940) seems sufficient to illustrate this conclusion.

In *Squalus* the conditions are similar to those in *Etmopterus*, but the ray-component is outlined very indistinctly and would scarcely have been suggested without a knowledge of the existence of such a ray-component in *Etmopterus*. In *Squalus* it is fused on to the lower portion of the outside of the hyomandibula. The levator muscle inserts itself into it at a 24—30 mm stage.

VOL. 24 1943

In *Scyllium* the lower end of the hyomandibula contains a large element not originally belonging to it. It forms the entire lower end of the hyomandibula. It is well differentiated from the medium portion of the hyomandibula, especially as it chondrifies later. The levator muscle of the hyomandibula inserts itself at the upper border of the lower end of the hyomandibula. During young stages the limit between the hyomandibula proper and the extra rudiment is much more conspicuous than at later stages, when perfect fusion takes place between the two parts.

The following are the theoretically most important results of the preceding discussion on the hyomandibula in sharks and rays:

A. in Sharks the hyomandibula consists of:
   a) a supra-pharyngohyale,
   b) an infra-pharyngohyale, present only in *Scyllium* and *Heterodontus* as the lower postspiracular ligament,
   c) an epal element, and
   d) a postspiracular mandibular branchial ray fused on to the epal element, in *Scyllium* forming the entire lower end of the hyomandibula.

B. in Rays the hyomandibula consists of:
   a) an epal element (the suprapharyngeal element is fused on to the neural skull and the infrapharyngeal is apparently absent),
   b) a postspiracular mandibular ray fused on to the hyomandibula.

In *Torpedo* this ray forms the "hooklike process". In *Potamotrygon* and *Mobula* the mandibular rays do not fuse on the hyomandibula. In *Raja* a lateral "ray" without relation to the hyomandibula is present.

THE HYOMANDIBULA IN TELEOSTOMIANS.

I begin this paragraph with the stating that the material on which the hyomandibular problem must still be based is the conditions in recent selachians, Chondrostei, Holostei and Teleostei, the development of the hyomandibula in *Polypterus* being too little known and that of the Dipnoi representing such peculiar conditions that even an embryonic existence of a hyomandibular element in *Protopterus* and *Lepidosiren* must be doubted and the possible hyomandibula in *Ceratodus* is liable to different interpretations. As the Holostei (*Amia, Lepidosteus*) have a hyomandibula which in principle is similar to that in teleosts, there are only three hyomandibular types upon which a hyomandibular theory could at present be founded, viz, that of Selachians, *Acipenser* and bony ganoids together with bony fishes. The place assumed by *Dipnoans* and *Polypterus* in the discussion must be but secondary owing to excessively wide gaps in our present knowledge.

A hyomandibular theory must be built up on evidence of an embryological and comparative anatomical nature and without any preconceived ideas. The relations between the hyomandibula and nerves, bloodvessels, ligaments and muscles are of course very important, but such relations may not be regarded as fixed once and for all. Experience seems to have taught us that the relations between skeletal elements and vessels and nerves may be variable even in related groups of fishes.

The different theories about the hyomandibular homologies are, with few exceptions, more or less constructive. Nerves, vessels, muscles and skeletal elements are mostly generally considered to have the same relations to each other, and then the problem has been to give such an explanation of the different hyomandibular parts as would be possible without changing these relations. It has accordingly been the fate of the hyomandibula to change, whereas the other structures have remained constant.

In teleosts that part which forms the anterior articular head of the hyomandibula anteriorly to the hyomandibularis VII foramen of that cartilage corresponds, according to ALLIS (1915), to the hooklike process of the hyomandibula of *Torpedo,* which he considers to be an interarcual cartilage between the hyal and mandibular arches. The development of the hooklike process, however, shows that this process is a postspiracular cartilage, i.e. a mandibular ray. The posterior articular head (behind the hyomandibular nerve) of the teleostean hyomandibula is said to be a hyal dorsal extrabranchial fused on to the upper part of the hyoid arch, which ALLIS considers to be a pharyngohyal, the interhyal (stylohyal) being the epal element. The primitive dorsal end of the pharyngeal element must have lost its contact with the neurocranium if there ever was one. Through the development of those dorsal

articular head elements the hyomandibula would then have acquired a new articulation on to the neurocranium dorsally to the jugular vein. Later (1918) he altered his opinion, and the anterior part of the hyomandibula was said to represent a branchial ray bar, the posterior part being derived from the dorsal extrabranchial of the hyoid arch.

In *Acipenser* and *Polyodon*, where there is no posterior articular head of the hyomandibula and where the hyomandibular nerve passes posteriorly to the hyomandibula, no extrabranchial component could be present. On the lateral side of the hyomandibula, however, there is in *Acipenser* a process projecting upward between the two branches of the hyomandibular nerve. BRIDGE (1879) found a small cartilaginous nucleus in *Acipens*er and a filament of cartilage in *Polyodon* which may represent the process. BRIDGE suggested that this cartilage was a rudimentary branchial ray. ALLIS (1915) stated that the process lies anteriorly to the ramus hyoideus facialis and not posteriorly to it, as it would normally if it were a ray of the related arch. He points out that it probably lies anteriorly to the afferent hyoid artery, which would not be normal for a branchial ray belonging to the hyoid arch. As it lies dorso-posteriorly to both the afferent and the efferent mandibular arteries it may be the extrabranchial of the mandibular arch. Thus the hyomandibular in *Acipenser* and *Polyodon* would consist of the pharyngohyal, an interarcual and the extrabranchial of the mandibular arch.

The hyomandibula of *Polypterus* bears the same relation to the hyoid and mandibular nerves as the process in *Acipenser*. ALLIS says: "If that little process were to undergo marked development, and the actual articular head of the hyomandibula to undergo a corresponding reduction, the hyomandibula of *Polypterus* would apparently arise."

EDGEWORTH (1926) has critizised ALLIS' views from the embryological standpoint and concludes his objections with the following words: "ALLIS' theory thus fails unless he can give evidence, in some developing Teleostome, of the existence of an originally separate procartilaginous or cartilaginous structure posterior to the r. hyomandibularis VII, which subsequently fuses with the hyomandibula."

SCHMALHAUSEN (1923) based his theory about the hyomandibula on the conditions in the hyal arch of *Ceratodus* and those of the branchial arches in *Acipenser*. As the morphology of the hyal arch in lungfishes has not yet got a well-founded explanation and as the interpretation of the different elements is still very uncertain, it is obvious that *Ceratodus* is very unsuitable as a starting-point for a hyomandibular theory. For this purpose the branchial arches of *Acipenser* are perhaps more useful. SCHMALHAUSEN describes the upper end of a typical branchial arch of *Acipenser* as consisting of an epibranchial, from which projects a stout medial process carrying a mediodorsally directed pharyngobranchial. The latter is said to articulate on to the

VOL. 24 1943

neurocranium medially to the jugular vein. On to the upper end of the epibranchial articulates a suprapharyngobranchial. The latter is said to abut on to the neurocranium laterally to the jugular vein. DE BEER (1925) says, however, that the pharyngeal elements in *Acipenser* both lie medially to the jugular vein, a statement which I can substantiate. SCHMALHAUSEN emphazises that the branchial arch has two branches, both of which articulate on to the neurocranium. In the hyal arch of selachians it must be the medial one of these branches that is present, in teleosts and ganoids it is the lateral. Compare: SÄVE-SÖDERBERGH, 1936). The erroneous interpretation of the relation between the jugular vein and the two branches in the branchial arches weakens this ingenious theory considerably. In the hyal arch of *Acipenser* a remnant of the medial pharyngohyal would be present as the cartilaginous bridge enclosing the jugular vein and the tractus hyomandibularis VII in a short canal. EDGEWORTH, however, says that the canal develops "by the formation and closing-in of a groove on the ventral surface of the auditory capsule, and is not due to the fusion of any pharyngohyal with the floor".

DE BEER (1924 a) starts from the "familiar fact that the mandibular arch frequently has two or more articulations with the neurocranium". Of these the "processus palatobasalis" is said to be the original tip of the arch. It lies medially to the jugular vein and to the nerves. The other, the processus oticus, articulates with the skull in a more lateral position; it lies external to the vein and to the nerves. As to origin, the processus oticus is probably, as ALLIS suggests, "a modified branchial ray". Now DE BEER asks whether the hyoid arch may not also have two articulations. He believes he has found such conditions in *Ceratodus*, where the "pharyngohyal" of SCHMALHAUSEN and others represents the basal and a blastema (strand of rudimentary procartilages laterally to the hyomandibular levator muscle the otic branch of the hyoid arch. EDGEWORTH, however, has stated that the said blastema is the developing rudiment of the opercular bone and the pharyngohyal the "oto-quadrate" cartilage, which forms the ventral wall of the jugular canal (HOLMGREN and STENSIÖ, 1936). Therefore the ideal hyoid arch of DE BEER is not found to be realized in any living animal, except *Chimaera* (HOLMGREN and STENSIÖ, 1939). According to DE BEER's theory the basal articulation is present in selachians, the otic in *Acipenser*. This otic articulation, he says, represents a modified branchial ray bar (DE BEER, 1925). In the Holostei "another branchial ray-bar" is "plastered" on to the hyomandibula from behind, and the hyomandibular nerve is thus enclosed between the two bars. The holostean condition cannot be derived from the selachian, whereas it can from the chondrostean. In *Polypterus*, according to DE BEER (1925), the otic articulation is also present, but "either the nerve or the cartilage has changed". The theories of DE BEER have been subjected to a close criticism by EDGEWORTH (1926).

a) *Some introductory remarks about the morphology of the hyomandibula.*

Considering the morphology of the mandibular arch, I have made an inventory of those structures which might be supposed to take some part in the formation of a mandibular arch constructed according to the branchial arch principles. A similar procedure might also be followed concerning the hyoid arch, and I might confine myself to a reference to page 50, but in dealing with the hyoid arch the problem seems to a certain degree to be simpler, as the hyoid arch has preserved more of the primary branchial arch characters than the mandibular.

It has been stated (DE BEER and MOY-THOMAS, 1935) that the only living fishes with a complete hyoid arch are the Holocephali. In *Callorhynchus* the upper half of the arch consists of an epal and a pharyngeal cartilage. In *Chimaera monstruosa* an infra- as well as a suprapharyngohyal are present as separate elements (HOLMGREN and STENSIÖ, 1936). To the epal element belongs a hyoid ray apparatus, in which the rays are connected basally, forming a basal cartilaginous plate. Thus to the epal element belongs a branchial ray bar, which might be said to contain an anterior plate-like part and a posterior part consisting of the rays. So far, however, this subdivision is quite formal. There is no symplectic part nor any extrabranchial present. The pharyngeal elements are somewhat insignificant structures. In sharks and rays the pharyngeal elements of the hyoid arch are reduced, the infrapharyngeal, if present at all, to a ligament, the suprapharyngeal in rays "plastered" on to the auditory capsule, in sharks fused on to the epihyal. A symplectic part is apparently always present, but extrahyal elements seem to be rare. A branchial ray bar, consisting of an anterior and a posterior part is generally present. In rays this anterior part forms the pseudohyal, in sharks it frequently forms plates on the edge of which the branchial rays are located.

Hyal extrabranchials are said to exist, for instance, in *Raja* and *Heterodontus*. Neither in *Raja* nor in *Heterodontus* could any extrahyals be found, the extrahyals described being the extrabranchials of the first or second branchial arch. But in others, as for instance in *Torpedo*, there are real extrahyals.

Thus those structures of the upper part of the elasmobranch hyoid arch which we might possibly expect to find in the teleostomian hyomandibula are: 1) the epihyal, 2) the infra- (interarcual) and suprapharyngohyals, 3) an anterior branchial bar portion (fused ray bases) and 4) a posterior branchial bar portion (branchial rays). To these elements may be added the symplectic. An extrahyal might also be supposed to be present, but as extrabranchials are not found in teleostomians, the existence of an extrahyal is not very probable.

From this survey it seems to follow that all theories in which selachian extrahyals are thought to take part in the formation of the teleostomian

hyomandibula, are plainly constructive. It is a matter of course that an extrabranchial of the mandibular arch could never be used for interpreting a hyomandibular structure, as there is no experience upon which the exsistence of such an extrabranchial, even in the mandibular arch, can be based.

b) *The location of the early rudiment of the hyomandibula and its relation to the principal head vein.*

In sharks and rays.

In sharks and rays the blastematic rudiment of the epal portion of the hyal arch develops exactly at the corresponding place, viz. immediately behind the spiracular canal at the very lining of the branchial cavity. In a 8—12.5 mm embryo of *Torpedo* the rudiment of the hyomandibula is found as a dense blastema lying immediately outside the epithelium of the cavity, and the same is the case in all other selachians investigated. Dorsally it reaches the medioventral wall of the headvein (vena capitis lateralis) and the upper part of the efferent hyoid artery, thus being separated from the auditory vesicle by a space filled in by a still undifferentiated mesenchymatic network with but few cells.

In an 8 mm embryo of *Torpedo* the rudiment of the levator hyomandibularis muscle being formed from the anterior end of the constrictor muscle sheath, abuts dorsally on to the *medioventral* wall of the headvein, where this vein broadens before sending off its anterior branch inside the hyomandibularis VII nerve. In fact the anterior end of the muscle rudiment is in contact with the base of this branch. Posteriorly where the headvein is as fine as thread, the hindmost part of the muscle rudiment lies *clearly medially* to the vein. Later, during ontogeny, the relations alter and the muscle comes laterally to the vein. In young embryos of *Scyllium, Squalus* and *Raja* the conditions are similar.

The early relations between the upper end of the hyoid arch, the levator muscle and the jugular vein thus differ somewhat from those in older embryos and in adult fishes. The upper end of the hyomandibula, in the early embryo ventromedially to the vein, later becomes medial, the levator muscle of the embryo, either medial or ventromedial to the vein, later becomes lateral. These shiftings seem to be correlated to the dorsal extension of the hyomandibula as its pharyngeal element develops and makes contact with the auditory capsule, whereby the vein and the muscle are pushed laterad.

In *Acipenser*.

In *Acipenser* the blastematic rudiment of the hyomandibula is found exactly in the place corresponding to that of the rudiment of the selachian:

immediately behind the spiracular canal rudiment and close to the lining epithelium of the branchial cavity. The upper part of the rudiment lies directly behind the spiracular canal and frontad following the curving of the canal without reaching beyond it dorsally. This dorsal part is flattened from in front and from behind, whereas the part lying ventrally to the canal is triangular in transsection. The upper end of the rudiment without a clearly defined boundary merges into the dorsolateral mesenchyme (of the pharyngeal element), which extends dorsad to the blastematic rudiment of the auditory capsule.

In an 8-day embryo of *A. ruthenus* the headvein lies straight dorsally to the hyomandibular rudiment. The rudiment of the musculus retractor hyomandibularis and opercularis (constrictor muscle, m. levator hyomandibulae) lies immediately ventrolaterally to the vein.

In an 8 mm embryo of *A. Gyldenstaedti* the relations of the hyomandibula and the jugular vein and the retractor muscle are in principle the same as in the early *ruthenus* embryo, but the headvein lies distinctly more laterally to the dorsal end of the hyomandibula and the muscle rudiment more medially in relation to the vein. These relations are about the same as those existing in early selachian embryos.

In a 10 mm *A. Gyldenstaedti* the vein lies straight dorsally to the upper end of the hyomandibular rudiment and the retractor muscle ventrolaterally and laterally to the vein. In an 11 day *Acipenser ruthenus* the vein lies somewhat medially to the hyomandibula and also medially to the retractor muscle.

In older embryos and in the adult, the head vein lies inside both the hyomandibula and the retractor muscle.

The initial circumstances governing the future development are thus essentially the same in *Acipenser* and in selachians, but the development then takes somewhat different ways. In selachians the hyomandibula gains contact with the otic capsule medially to the vein, in *Acipenser* laterally to the vein. In fact, a shifting of the relations is apparent in both cases.

In *Amia*.

In a 6.5 mm embryo of *Amia* the head-vein lies straight dorsally to the lateral surface of the hyomandibular rudiment. In a 7 mm embryo it lies straight dorsally to the rudiment of the anterior part of the hyomandibula, and at a 7.5 mm stage it is found dorsally to the medial surface of the same part. The upper anterior part of the constrictor muscle sheet in the 6.5 mm embryo reaches the ventral or ventrolateral wall of the vein dorsally.

VOL. 24 1943

c) *The early relations of the rudiment of the hyomandibula to the hyomandibular nerve.*

In *Selachians.*

In selachians the hyomandibular ganglion always lies anteriorly to the rudiment of the hyomandibula and at early stages the nerve lies laterally to the rudiment. The nerve thus runs frontally to the rudiment.

In *Acipenser.*

In an 8 mm *Acipenser Gyldenstaedti* the hyomandibular ganglion lies immediately frontally to the upper end of the hyomandibular rudiment but clearly dorsally to it. The hyomandibular nerve from the ganglion goes almost directly ventrad outside and a little in front of the anterior border of the hyomandibula. In a 10 mm embryo the same is clearly seen, but here the nerve makes a bend caudad and laterad to pass laterally to the hyomandibula. In later embryos the hyomandibular ganglion lies a little more frontally and the bend of the nerve is greater. As the hyomandibula now developes dorsad to attain the auditory capsule this growth takes place outside and in front of the bent part of the nerve and thus the nerve takes its course dorsally inside, ventrally outside the hyomandibula. *Acipenser ruthenus* behaves in identically the same manner.

Thus there occurs in *Acipenser* a shifting of the relation of the hyomandibular nerve to the hyomandibula. At early stages the nerve is found laterally to the frontal border of the hyomandibula, as in selachian embryos and in adult selachians, at later stages its dorsal part lies medially to the hyomandibula, whereas the ventral part lies in a position laterally to it.

In *Amia.*

In my 6.5 mm embryos of *Amia* the hyomandibular nerve coming from its ganglion passes dorsally to the hyomandibular rudiment and then bends ventrad on the outside of it. At the 7 mm stage it is seen in the same position but during this stage a dorsal portion of the hyomandibula has formed which is connected with the early rudiment of the hyomandibula in front of the nerve. The nerve thus runs posteriorly to the hyomandibula, to become somewhat later enclosed in a foramen piercing the hyomandibula. At the 7 mm stage the relation between the hyomandibula and the nerve is as in the adult *Acipenser.*

In *Lepidosteus.*

In the youngest stage of *Lepidosteus* described by HAMMARBERG and at my disposal, the relation between the hyomandibula and the nerve is as in the 7 mm *Amia.* Later the relation is the same as in older *Amia* embryos.

In *Salmo.*

The relation between the hyomandibular nerve in early embryo of *Salmo* is as in early embryos of *Amia* and later corresponds to that in older embryos.

The change in relations between the hyomandibular nerve and the hyomandibula occuring in the teleostomian fishes during their development depends upon the mode of development of the hyomandibula, which will be considered in the next paragraph.

d) *The development of the hyomandibula in Teleostomians.*

In *Acipenser.*

VOL. 24 1943

The early rudiment of the hyomandibula in *Acipenser* (*ruthenus, stellatus* and *Gyldenstaedti*) lies immediately behind the high and flat epithelial mass representing the spiracular canal. It follows the curving outline of the canal without coming dorsally to it. A structure of considerable interest, and to my knowledge not noticed before in the blastematic rudiment of the hyomandibula, is a very well developed medial process on its upper end behind the spiracular rudiment. This process (fig. 39) projects in a medial direction following the inner layer of the entoderm of the roof of the branchial cavity. It extends rather far mediad below the hyoid artery as the latter takes its transverse course. This blastematic process is pointed medially. It is very distinctly seen in 7—8 day embryos of *Acipenser ruthenus* and in 9—13 mm embryos of *A. Gyldenstaedti* and *stellatus*. In the younger stages there is no line of demarcation between the hyomandibula and its process. Later, however, when at the 10 day stage the hyomandibula becomes prochondrial, the process is always blastematic and becomes less dense than before. At length (29 day stage) it develops into a ligament extending from the articular head of the hyomandibula in a medial direction, to end in the connective tissue (fascia) below the basis cranii dorsally to the spiracular canal and posteriorly to the hyoid artery. Outside the upper

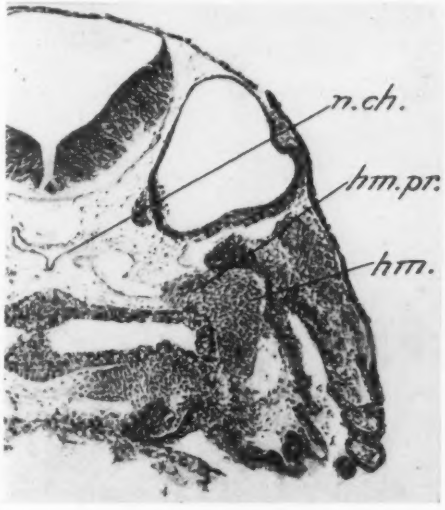


Fig. 39. *Acipenser ruthenus.* Embryo 7—8 days old. Transverse section level with the *hyomandibula.*

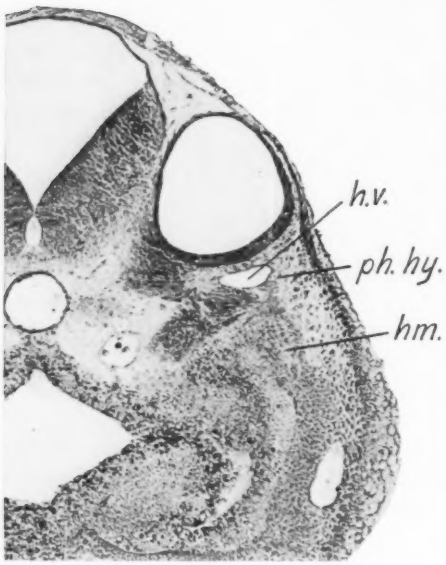


Fig. 40. *Acipenser ruthenus*. Embryo about 9 mm, somewhat older than that in fig. 39. Transversal section, level with the hyomandibula.

part of the rudiment of the hyomandibula there lies a rather scattered mesenchyme, which dorsally enters between the upper end of the rudiment and the blastematic auditory capsule. In this mesenchyme at the 9 mm stage of *Acipenser ruthenus* a denser portion has arisen. This portion forms a blastematic band connecting the upper end of the foremost portion of the hyomandibula with the auditory capsule (fig. 40 ph.hy.). It lies in front of the hyomandibular nerve and outside the head vein. Through this blastema the hyomandibular nerve acquires its definitive position in relation to the hyomandibula. Connected with this dorsal blastema is a blastematic strand (fig. 41 op.pr.) extending some distance along the anterolateral part of the hyomandibula. This strand, probably representing a ray-bar, detaches from the dorsal blastema and forms the rudiment of the small process (opercular process) of the hyomandibula. In *Acipenser Gyldenstaedti* this ventral blastema is far larger than in *A. ruthenus* and here forms the cartilage that overbridges the hyomandibular nerves in *A. Gyldenstaedti* (HOLMGREN and STENSIÖ, 1936).

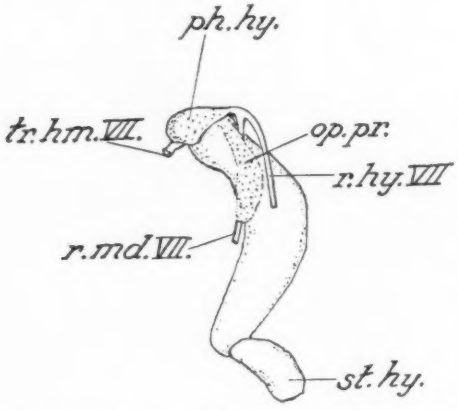


Fig. 41. *Acipenser Gyldenstaedti*. Embryo 14 mm. Hyomandibular, reconstruction, lateral view.

The description of the development given above makes it certain that in the upper part of the hyomandibula of *Acipenser* there are four structures to be taken into account when determining the morphological value of it: the main part (epihyal), the medial process, the dorsofrontal blastema and the opercular process (ray-bar) blastema. To determine the morphological value of these parts it is necessary to compare them with the development of an anterior

branchial arch in *Acipenser,* as described on p. 46.

If we compare the development of the branchial arch with the hyoid arch there can hardly be any doubt that the main part of the hyomandibula is the epal element of the hyoid arch. The "infrapharyngohyal" is represented by the medial process of the epihyal, this process later transforming into a ligament and the suprapharyngohyal by the dorsal blastematic mass described above. This blastema in an 11 day embryo is clearly seen as a pad of prochondrial tissue lying between the epihyal and the auditory capsule (fig. 42). Medially it extends below the head vein, which is partly enclosed in a groove on the ventral side of the otic capsule. The pharyngohyal is thus fused on to the capsule and takes part in the formation of the posterior part of the floor of the jugular canal (fig. 43). This canal, then, is not, as EDGEWORTH says, formed by the auditory capsule growing down around the vein. The pharyngohyal, one part of which is probably fused on to the hyomandibula and another on to the otic capsule, later becomes flattened out and finally the only visible remainder of it forms the thin articular disc on to which the hyomandibula articulates. The lateral blastematic strand mentioned in the preceding lines, represents the opercular process and may be regarded a raybar more or less strongly fusing on to the epihyal.

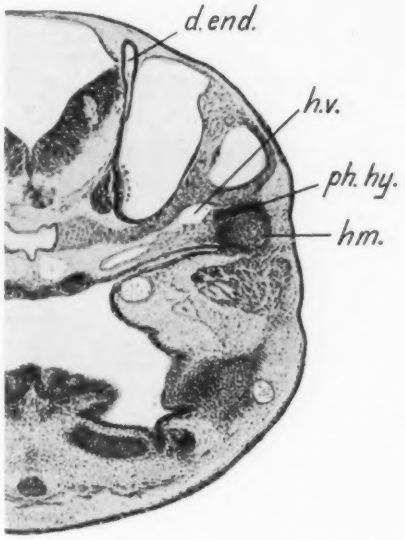


Fig. 42. *Acipenser ruthenus.* Embryo 11 days. Transversal sections through the level of the upper end of the hyomandibula.

The comparison between the upper part of the hyal arch and the first branchial arch in *Acipenser* has thus afforded strong evidence in support of the theory that the hyomandibula is chiefly an epihyal element as it is in rays and to a considerable extent also in sharks.

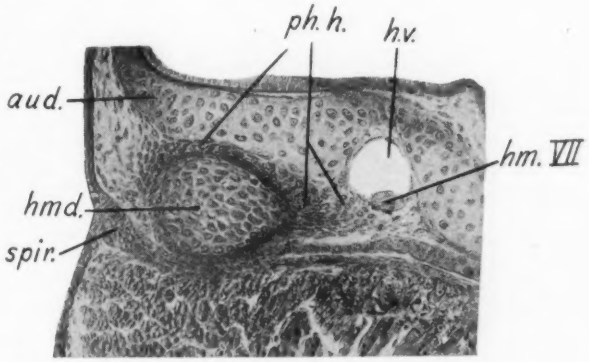


Fig. 43. *Acipenser ruthenus.* Embryo 12.5 mm. Transversal section through the upper end of the hyomandibula.

In *Amia*.

During the youngest stages at which the hyomandibula has been described in *Amia* it is already pierced by the hyomandibular nerve. Concerning his stages 1 and 2 (7.5—8 mm) PEHRSON (1922) only says: "The frontal edge of the hyomandibula is excavated to make room for the spiracular rudiment. The hyomandibula is pierced by the ramus hyoideomandibularis of the nervus facialis."

In a 6.5 mm embryo of *Amia* the rudiment of the hyomandibula consists of a blastema lying close to the lining epithelium of the branchial cavity. Dorsally its frontal surface reaches to a level with the lower margin of the posterior side of the rudiment of the spiracular canal. The hyomandibular nerve runs outside the hyomandibular rudiment, i.e. morphologically frontally to the rudiment.

At the 7 mm stage a new rudiment has arisen, viz. a separate blastema lying immediately behind the spiracular rudiment and extending laterad, so that the rudiment reaches the skin epithelium behind the spiracle. The hyomandibular nerve goes between this new rudiment and the upper end of the hyomandibular rudiment of the preceding stage.

At the 7.5 mm stage the two rudiments have fused anteriorly to the hyomandibular nerve. Thus the nerve, having come from its ganglion, proceeds ventrally to the second rudiment and caudally to the first rudiment of the hyomandibula, occupying a position corresponding to that in *Acipenser*. There could scarcely be any doubt, therefore, that the *Amia* hyomandibula of the 7.5 mm stage is composed of an epihyal and a suprapharyngohyal. An infrapharyngohyal is represented by a medial process of the epihyal extending below the efferent hyoid artery, as in *Acipenser*.

At the 8 mm stage the upper part of the hyomandibula (the suprapharyngohyal) is about to reach the auditory capsule, and the nerve pierces the hyomandibula. How this perforation arises I could not determine with any certainty, but it seems not impossible that the enclosing process is effected by the addition of a branchial ray-bar to the posterior border of the early rudiment. Some of my sections seem to demonstrate that the posterior part of the hyomandibula (posterior to the nerve foramen) is composed of at least two horizontally located raylike prochondrial bars, of which the lower runs out into the future opercular process. The posterior ends of these bars are blastematic and the blastema of the opercular process extends to the lateral surface of the opercular fold, probably there forming the first rudiment of the opercular bone. Another argument in favour of the presence of a branchial bar portion, is the fact that there is a narrow zone immediately behind the

VOL
24
194

nerve foramen, where the skeletal tissue is less advanced than it is further caudally.

The development of the hyomandibula of *Amia* as far as could be observed from the avaiable material, tallies fairly well with that of *Salmo* (se p. 88).

In *Lepidosteus*.

The youngest hyomandibula described in literature belongs to a 9.3 mm specimen in the collections of the Zootomical Institute. Of the hyoid arch of this *Lepidosteus* embryo HAMMARBERG (1937) says: "Caudal von den Anlagen des Mandibularbogens liegt die, wie aus den folgenden Stadien hervorgeht, einheitliche Anlage der ventralen Elemente des Hyoidbogens, Stylo-, Cerato- und Hypohyale. Sie liegt mit ihrem Ventralteil ganz ventral, wo der Mundboden in die Seitenwand der Mundhöhle umbiegt, und erstreckt sich von diesem Punkt in beinahe dorsaler Richtung in der Seitenwand hinauf. Caudal von ihrer oberen Spitze liegt das hyomandibulare Blastem, dessen Form, die aus den Fig. 1 und 2 deutlich hervorgeht, dahin zu deuten scheint, dass es einen Processus symplecticus in sich schliesst. Von den Seite gesehen, ist es winkelig gebogen, und in diesem Winkel passiert der Truncus hyoideomandibularis. Der Nerv nimmt eine verticale Lage medial von dem Hyomandibulare ein, biegt an dessen Unterrand in dem Winkel nach vorn und ausen um, und lateral von dem Knorpel setzt er sich rostroventralwärts fort. Etwas vor der Mitte dieses Blastems erstreckt sich die Anlage des Recessus dorsalis des Spritzlochkanals dorsorostral von demselben nach aussen." This description corresponds closely to the series of sections of the 9.3 mm stage. It might be remarked, however, that the lower end of the hyomandibula is connected by a blastematic strand with the upper end of the lower portion of the hyoid arch. As in this specimen there is a slender medial blastematic process at the middle of the rudiment entering between the lining epithelium of the branchial cavity and the efferent hyoid artery, and as the upper part of the hyomandibula arises in a dorsolateral position in relation to the lower part, it is obvious that this hyomandibula corresponds perfectly to that of a 7.5 mm embryo of *Amia*. According to our interpretation of this stage of *Amia*, the hyomandibula of the 9.3 mm *Lepidosteus* consists of an epihyal, an infrapharyngohyal and a suprapharyngohyal.

In a 10.1 mm *Lepidosteus* embryo the hyomandibular nerve has just become enclosed, a slender blastematic band being formed behind the nerve connecting the upper part of the hyomandibula with the lower. The enclosing process here could hardly be described as a surrounding of the nerve by the caudal growth of the hyomandibular portion anteriorly to the nerve.

At the 11 mm stage the essentials of the hyomandibula are already formed. HAMMARBERG describes it as follows: "Durch Vorknorpel ist das Hyomandi-

VOL. 24 1943

bulare mit dem ventraloteralen Rand der Labyrinthkapsel verbunden. Nahe seinem caudoventralen Rand ist der Truncus hyoideomandibularis von dem Hyomandibulare umwachsen. Die Ränder des Hyomandibulare gehen ohne scharfe Grenzen in das umgebende Mesenchymmasse über, während die lateralen und medialen Flächen wohl abgegrenzt sind." To this description may be added the fact that from the constrictor muscle there has already branched off an opercular portion which ends in a blastema covering the upper caudal end of the hyomandibula, which may thus be considered to contain a processus opercularis. At the lower posterior corner of the hyomandibula a similar blastema is present, representing the processus subopercularis (HAMMARBERG). Along the caudal border of the hyomandibula a blastematic band[1] connects the two opercular processes with one another. The processus subopercularis certainly arises from a separate blastema and at the 18.7 mm stage chondrifies separately. HAMMARBERG considered it to be a rudimentary branchial ray belonging to the hyoid arch. If this were the case, there would be no serious objection to the opercular process also being regarded as a similar ray. Thus the postforaminal portion of the hyomandibula may be a hyoid branchial ray bar fused on to the hyomandibula.

In *Polypterus*.

The earliest development of the hyoid arch is very little known. MOY-THOMAS (1933) gave figures for a 6.75 mm stage. The hyosymplectic there is a very long narrow prochondrial band extending downwards from the auditory capsule. As the mid-portion of this band lies on a level with the future mandibular articulation, there seems to be some doubt as to its morphological nature. It might perhaps represent the entire arch. A comparison between this stage and the 8 mm stage seems to support such an assumption. The two stages have so little in common that it is hardly possible to compare them. There are 4 branchial arches in the 6.75 mm, 3 in the 8 mm and 4 in the 9.3 mm stage. A new investigation of this precious material is strongly indicated, especially as EDGEWORTH's reconstructions of the same embryos (8 mm and 9.3 mm) tally very little with those of MOY-THOMAS.

In *Salmo*.

The early development of the hyomandibula is very little known in Teleosts. EDGEWORTH (1926), however, describes three stages of *Salmo trutta* of 5, 5.5 and 6.5 mm respectively. Of the 5 mm stage he says that: "the hyoid bar lies immediately behind the 1st gill-cleft. The tr. hyomandibularis VII passes

[1] This band, however, is not regarded with any certainty as being an organized connection between the two processes, but might consist of the posterior blastematic border of the growing hyomandibula only.

outwards behind the hyomandibula, which does not extend behind the nerve". In the 5.5 mm embryo the hyomandibula extends backwards "forming a plate, and the tr. hyomandibularis VII perforates it". In the 6.5 mm embryo chondrification has taken place. There is nothing to signify that it has been formed by the coalescence of two skeletal elements. It should here be pointed out that in the 5.5 mm stage the postnerval part of the hyomandibula lies near the skin epithelium and the prenerval near the epithelium of the branchial cavity. In the figure of the 5 mm stage no postnerval portion is visible. EDGEWORTH says: "No evidence was seen, either in the embryos depicted or in others of the same stages, that the development of the plate is due to the fusion of any additional cartilage. Its formation is simply due to backward growth or extension of the first-formed portion of the bar."

DE BEER has given some data regarding the development of the hyomandibula in *Salmo fario*. His description, however, concerns only the chondrified or chondrifying parts of the hyomandibula, omitting blastematic or early prochondrial parts of the rudiment. As those parts seem to be of decisive importance for the solution of the hyomandibular problem, no conclusions can be drawn from the descriptions. Of great interest, however, is his statement that at a certain stage (8 days before hatching, embryo 9.4 mm long) the upper end of the hyomandibula is cleft, the hyomandibular nerve passing through the notch. Later (7 days before hatching, 10.4 mm embryo) this notch closes to form a foramen. This description appears to correspond fairly closely to the findings of G. T. D. HENDERSON, quoted by EDGEWORTH (1935), according to which the hyomandibular nerve in *Galeichthys* passing in front of the hyomandibula sinks into a notch in the anterior edge of the hyomandibula. The same is also said to be the case in *Amiurus*. In other Teleosti the nerve is said to sink in from behind.

My researches into a very rich material of *Salmo salar* have yielded other results, as will be described in the following pages.

In *Salmo salar* the differentiation of the hyoid arch begins during 15—16 day (at 10—12° C.) stages of development (or somewhat earlier) and is practically concluded in 25—27 day embryos.

In a 16-day embryo of *Salmo salar* the hyal arch is filled (except the rudiment of the muscles) up with cell masses, extending from the spiracular canal in front of the anterior part of the blastematic rudiment of the auditory capsule dorsally and to the ventral side of the entodermal gut ventrally, containing the primordia of the entire skeletal arch. The dorsal part of these is destined to form the hyomandibula and the operculum. (At this stage the myoblasts and the mesenchymatic cells are not well differentiated in the sections). In the dorsal part two distinct cell-groups are discernible. Behind the medial part of the spiracular canal, and following its curvature and that of the branchial intestine, there is a strong blastema located perpendicularly. The cells of this

VOL. 24 1943

blastema which border upon the epithelium of the spiracular canal and the branchial cavity are arranged with the longitudinal axes of their nuclei perpendicular to it. Ventrally, this part of the rudiment is continuous with that of the future ceratohyal. The other blastema is found to be caudally and dorsally to the lateral half of the spiracular canal. Dorsally it extends to the auditory capsule rudiment and caudo-laterally to the skin of the opercular fold. Its cells are irregularly arranged. Medially this blastema is rather well delimited from the medial blastematic mass just described. The boundary line is still more pronounced by the course of the hyomandibular nerve. This nerve turns straight laterad from the hyomandibular ganglion. It passes the medial blastema dorsally and at its anterodorsal border. The nerve then runs between the two blastemas, accentuing their structural limit still more.

At the 20 days 5 hours stage, the future shape of the hyomandibula is already outlined and the muscle rudiments well differentiated (fig. 44 a). At this stage the hyomandibula consists of an antero-ventral and a postero-dorsal part, structurally well demarcated from one another. Between the two parts runs the hyomandibular nerve, laterally to the ventral part and medially to the dorsolateral. The ventral part has an antero-ventral and a postero-ventral process. The former is the processus symplecticus, the latter the rudiment of the stylohyal. *In front of and medially to the stylohyal rudiment the hyomandibular rudiment is broadly connected with the ceratohyal, and the stylohyal lies laterally to this connecting bridge.* It thus belongs to a level that is superficial in relation to the blastema connecting the hyomandibula and ceratohyal proper. This connecting blastema is rather thin in front of the rudiment of the stylohyal. The posterior margin of the postero-dorsal portion of the hyomandibular blastema extends towards the skin of the opercular fold.

The 22 days 7 ½ hours stage differs in no other respect from the preceding than that the dorsal portion of the hyomandibula has fused with the ventral anteriorly and posteriorly to the hyomandibular nerve (fig. 44 b).

The hyomandibular nerve has thus become enclosed in a canal piercing the hyomandibula. The fusion of the two blastemas of the hyomandibula is so intimate that the structural boundary between them could not be determined in detail. It seems possible therefore that already at this stage cells belonging to the dorsal blastema are present between the nerve and the ventral blastema. In that case the nerve would pierce the dorsal blastema at its lower border.

In an embryo of 23 days 7.5 hours prochondrium has developed. As a result of this development the boundary between the dorsal part of the hyomandibula and the ventral has become very much accentuated. In this embryo it is clearly discernible that the hyomandibular nerve passes between the two parts. The stylohyal is connected with the hyomandibula as at the 20-day stage.

In an embryo of 24 day 4.5 hours (10—12° C.) chondrification is beginning. There is an unchondrified zone left between the two parts of the hyomandibula. On the right side the hyomandibular nerve runs just dorsally to this zone, while on the left it pierces the dorsal portion of the hyomandibula.

In the right hyomandibula of an embryo of 25 days 6.5 hours (fig. 44 c) the dorsal portion is pierced by the hyomandibular nerve, whereas in the left

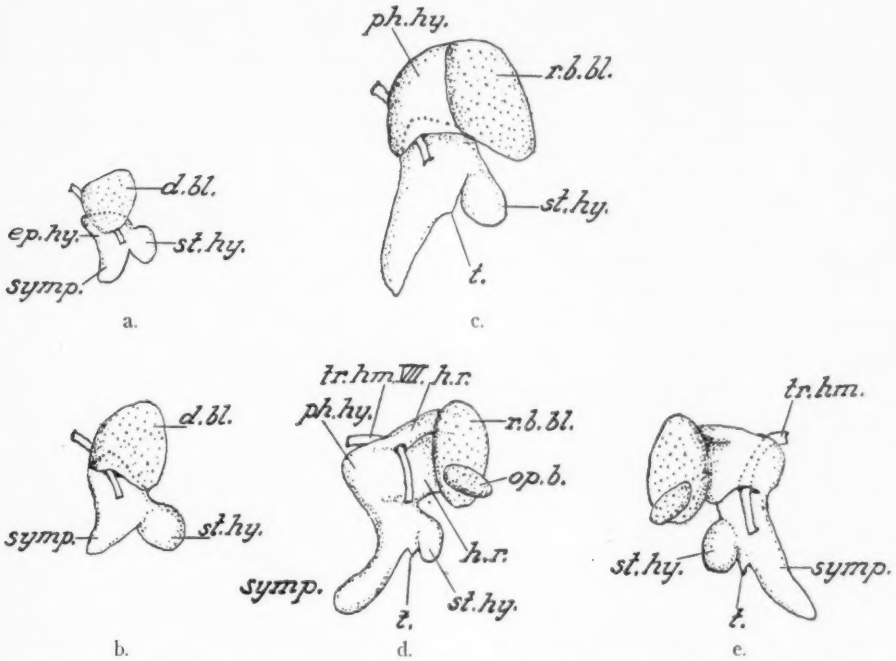


Fig. 44. *Salmo salar*. A series of reconstructions of the hyomandibula a) of embryo 20 days 5 hours, b) 22 days 7.5 hours, c) 25 days 6.5 hours, d) and e) 26 days 1.5 hours, left and right hyomandibula.

hyomandibula the nerve pierces at the boundary between the two portions. At this stage the chondrification is fairly strong. In the blastematic connection described above between the hyomandibula and the ceratohyal chondrification has taken place in its anterior thinner portion. In this portion cartilage has developed in connection with the hyomandibula, forming a slender tip of cartilage immediately in front of the stylohyal. The stylohyal lies as it did earlier and is connected with the hyomandibula and the ceratohyal respectively by the rest of the primary connecting bridge between the two latter parts.

In a 26 days 13.5 hours embryo on the left side *a cartilage thread corresponding to the cartilages tip of the preceding embryo connects the hyomandibula directly with the ceratohyal anteriorly to the stylohyal*. On the right side a cartilaginous tip is present as in the preceding stage and in older em-

bryos. In this embryo the hyomandibular nerve pierces the dorsal portion of the hyomandibula but at different levels on the two sides.

In a 26 days 17 hours stage the right hyomandibular nerve runs between the dorsal and ventral portions of the hyomandibula, the left pierces the dorsal portion.

In an embryo of 28 days 17 hours the right hyomandibular nerve runs through a notch[1] in the most dorsal still prochondrial border of the dorsal portion of the hyomandibula[2], whereas the left nerve runs between the two portions. Thus the right nerve practically runs dorsally to both hyomandibular portions.

An embryo of 26 days 1.5 hours is of great interest as it demonstrates a variation which may afford some explanation of the dorsal part of this hyomandibula (fig. 44 d). On the left side the nerve runs but a very short distance ventrally to the dorsal edge of the hyomandibula, thus piercing the hyomandibula very high up dorsally; on the right side the nerve foramen lies but a short distance dorsally to the boundary between the two parts of the hyomandibula. On the left side it is very distinctly seen that that part of the hyomandibula which lies behind the nerve foramen is composed of a dorsal and a ventral part. The dorsal forms a prochondrial cylindrical rod along the dorsal margin of the hyomandibula. The ventral portion is broader than the dorsal and flattened from the sides. Caudally the two parts merge into a big blastema, filling up an anterior part of the opercular fold. The ventral part runs out towards the rudiment of the opercular bone (op.), represented in the actual embryo by a superficial condensation of the blastema of the opercular fold. On the right side, in spite of the different location of the hyomandibular foramen, the same hyomandibular portions are discernible, but not so distinctly as on the left side. The attention having once been drawn to these conditions, it was possible to recognize them in other embryos in which chondrification of the upper part of the hyomandibula was beginning.

In an embryo 27 days 7.5 hours old the development of the hyomandibula is more advanced than in the others. At this stage the operculum is developing, as some insignificant bone lamellae are seen in the interior of a blastematic condensation, corresponding to that mentioned in the preceding stages.

Thanks to the descriptions of these different stages it is now possible to make out the general traits of the development and morphology of the hyomandibula of *Salmo salar*.

1) In the earliest examined embryos the hyal arch consists of an arch of mesenchymatic tissue extending from the spiracular canal dorsally around the

[1] Dorsally this notch is joined to a foramen by not yet prochondrial tissue belonging to the dorsal hyomandibular portion.

[2] This case corresponds to that recorded by DE BEER, in which the hyomandibular nerve runs through a notch at the upper border of the hyomandibula.

entodermal gut close to its lining epithelium. It contains the continuous primordium of the epihyal, ceratohyal and hypohyal. Added to this is a dorsal blastematic portion lying dorsolaterally to the upper end of this arch. This dorsal mesenchyme forms the upper part of the hyomandibula. The hyomandibular nerve runs dorsally and laterally to the mesenchymatic arch and becomes enclosed in a canal of variable location as soon as the dorsolateral mesenchyme joins the arch mesenchyme.

2) The dorsal mesenchyme forms the suprapharyngohyal (anterior articular head) of the hyoid skeletal arch anteriorly to the hyomandibular foramen. Posteriorly to the foramen two horizontal skeletal bars are formed, of which the dorsal is in contact with the auditory capsule forming the posterior articular head. The ventral runs out into the opercular process and also furnishes the blastema in which the opercular bone develops.

3) The arch blastema of paragraph 1 becomes segmented as chondrification begins in an upper part, representing an epihyalo-symplectic and a lower part —the ceratohyal + hypohyal. These parts remain, however, in broad blastematic connection with each other. The rudiment of the stylohyal is connected with the lateral surface of this connecting bridge. This rudiment extends laterally almost to the skin epithelium. It thus belongs to a more superficial layer than the hyoid arch proper. As the lower part of the hyomandibula chondrifies it may occur that a direct cartilaginous connection between the hyomandibula and the ceratohyal arises in the anterior part of the primary connecting bridge, whereas that part of this bridge to which the stylohyal is connected remains mesenchymatic. In such a case the hyomandibula is joined on to the ceratohyal by two connections, one anterior cartilaginous and one posterior mesenchymatic. To the latter the stylohyal is attached laterally. Later the mesenchymatic part chondrifies together with the primary stylohyal.

4) A symplectic process is always present on the anteroventral border of the lower part of the hyomandibula.

The hyomandibula of *Salmo salar* may thus be considered to consist of:

1) a suprapharyngohyal forming the anterior articular head and carrying (at least) 2 rays, the upper forming the posterior articular head, the lower the opercular process,

2) an epihyalo-symplectic forming the lower part of the hyomandibula,

3) the hyomandibula has a primary blastematic connection with the ceratohyal. An anterior part of this connection casually may chondrify. The posterior part forms the ligaments connecting the hyomandibula with the ceratohyal. Tre stylohyal is probably a branchial ray belonging to this connection. (See p. 104). It is not possible to decide whether this ray belongs to the epihyalosymplectic or to the ceratohyal.

The great variability in the position of the hyomandibular nerve in relation to the elements of the hyomandibula seems to be of considerable interest as

VOL. 24 1943

it demonstrates that during early development the relations are not firmly established as might be thought from the fact that in the adult no corresponding variations are known. The variations described in the preceding pages are due to these embryos being reared in the laboratory in running tap-water of higher temperature than under normal conditions. In a set of embryos reared in lower temperature and in water from a lake the variations were very rare, the nerve in those embryos piercing the hyomandibula but a little distance dorsally to its ventral portion.

e) *The "hyomandibular muscle".*

SEWERTZOFF (1923) has stated that the musculus levator hyomandibulae on the outside of the hyomandibula of Selachians develops from the hyoid muscle plate. He also (1928) stated that the protractor muscle on the inside of the hyomandibula of *Acipenser* is derived from the mandibular muscle plate, whereas the so-called retractor muscle on the outside is formed from the hyoid muscle plate. These findings were confirmed by EDGEWORTH (1935), who added that the protractor muscle is innervated partly at least from the trigeminal, the other from the hyomandibular nerve. In *Amia, Lepidosteus* and *Salmo* the conditions are as in *Acipenser,* but the protractor muscle lies more inside the hyomandibula. The so-called "hyomandibular muscle" of DE BEER (1924) is thus different in Selachians and in Teleostomians. The different position of this muscle in regard to the course of the head-vein in Selachians and Teleostomians is therefore of no value to the hyomandibular theory.

f) *The hyomandibula in Polypterus and Palaeoniscids.*

The general shape of the hyomandibula of *Polypterus* is too well-known as to need any new description. I refer therefore only to the excellent pictures published by ALLIS (1922). Here I will consider only a few points concerning its probable composition. As there is no early embryological material available, it is not possible to tell whether this hyomandibula, like that of other Teleostomians, contains any pharyngeal element. This important question thus must be left without any answer.

In my youngest specimen, 37 mm long, belonging to *Polypterus senegalus,* the upper end of the hyomandibula is broad as in *P. bichir* (ALLIS, 1922), consisting of two confluent articular heads, the anterior of which is thicker than the more flattened posterior. The articular groove is placed with its longer axis at about 45 ° to the longitudinal axis of the skull. The anterior part of the articular part of the hyomandibula is thus situated much lower than the posterior part, which attains the lateral crista of the auditory capsule

and moreover reaches the lateral border of the parietal bone to which it is attached.

The medium part of the epihyal (hyomandibula) is covered by a thin layer of perichondrial bone. This layer thins out towards its upper and lower ends, leaving these parts uncovered. The posterior part of the upper end (posterior articular head) of the hyomandibula is ossifying without being

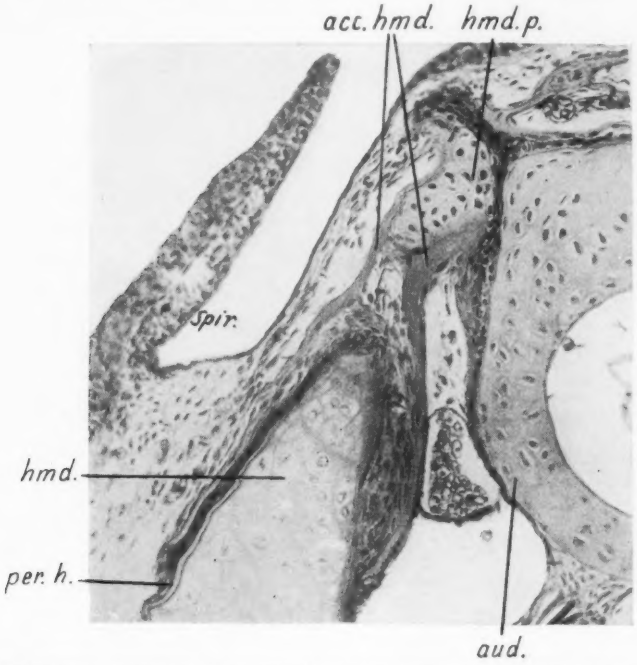


Fig. 45. *Polypterus senegalus*. Transversal section through the posterior part of the hyomandibula. *acc.hmd.*, accessory hyomandibula; *aud.*, auditory capsule; *hmd.*, hyomandibula; *hmd.p.*, posterior articular head of hmd.; *per.h.*, perichondrial bone of hmd.

connected with the bone cylindre of the epihyal. This bone is also perichondrial and considerably thicker than that of the epihyal. The behaviour of the articular head bone is very peculiar as it has developed a bone lamella which has grown down outside the epihyal bone (fig. 45), covering the caudal part of its lateral surface and part of its caudal border a distance towards the opercular process. This lamella could easily be mistaken for a dermal bone lamella, what it cannot be because it has grown out from a distinct and typical perichondrial bone.

In preceding lines I have already antecipated that the main part of the hyomandibula is an epihyal, an antecipation which is evidenced by the presence on it of the opercular process, which is connected with the epihyal. The fact that the posterior "articular head" of the hyomandibula ossifies independently of the rest of the hyomandibula undoubtedly means that it has an independent

origin. What might it be then? Either is it a pharyngohyal or a part of a ray-bar plastered on to the hyomandibula, the rest of the bar being the opercular process.

ALLIS (1918 and 1922) has given strong reason supporting the latter alternative. He has already stated that the "accessory hyomandibula" contains a bit of cartilage, a fact which is decidedly against the supposition that it should be a bone belonging to the series of spiracular ossicles as supposed by WIJHE (1882), BRIDGE (1888), POLLARD (1891, 1892) and HOLMGREN and STENSIÖ (1936), the two latter authors having overlooked ALLIS' statement. ALLIS' considers the "bit of cartilage to represent the dorsal end of the bar formed by the fusion of the bases of the posterior row of branchial rays of the hyal arch, here not completely differentiated to form a posterior articular head of the hyomandibula such as is found in the Teleostei. The ligament that connects the bone that encloses this cartilage with the opercular process represents the ventral portion of the posterior articular head of the teleostean hyomandibula, and the shank of the accessory hyomandibula is a secondary formation of membrane origin." With exception of the "membrane origin" of the shank of the accessory hyomandibula I accept fully the interpretation of ALLIS quoted in the preceding lines.

In 1936 NIELSEN considered the accessory hyomandibula to have originated "from fused tooth plates on the hyomandibula in a similar way to what is generally the case with dermal bones related to the visceral skeleton". This interpretation cannot be sustained, if we consider that the bone in question is perichondrial. Also the terme "dermohyal" introduced by NIELSEN (1942) is not appropriate from the same reason. An accessory hyomandibula ("dermohyal") was probably present in many Palaeoniscids, as stated by NIELSEN.

If the main part of the hyomandibula is the epihyal element of the hyal arch, then it is clear that the next element of the arch cannot be an epihyal (of ALLIS) but must be a stylohyal (interhyal) (EDGEWORTH) that connects up the epihyal with the ceratohyal as in ganoids and teleosts.

The hyomandibula of *Polypterus* has been considered a hyo-symplectic for instance by EDGEWORTH. The symplectic element, however, is very insignificant, represented in my 37 mm embryo by a small anterior process on the lower end of the hyomandibula. This process extends towards the palato-quadrate on the outside of the posterior lower corner of the palato-quadrate cartilage.

In the Palaeoniscids the hyomandibula is principally as in *Polypterus*. In some Palaeoniscids as *Boreosomus* (NIELSEN, 1942), *Ospia* (STENSIÖ, 1932), *Broughia* (STENSIÖ, 1932) the opercular process was well developed, in others as *Palaeoniscus, Nematoptychius, Oxygnathus, Elonichthys, Cycloptychius, Amblypterus* (TRAQUAIR, 1877), *Cheirodus* (TRAQUAIR, 1879),

Saurichthys (STENSIÖ, 1925), *Gyrolepis* (1921), *Australosomus* (STENSIÖ, 1932), *Glaucolepis* (NIELSEN, 1942) and others it was missing or was reduced to a corner at the posterior border of the hyomandibula. Disregarding the more or less developed opercular process in Palaeoniscids, these fishes had a hyomandibula so very much reminding of that in *Polypterus*, that it is impossible not to regard their hyomandibula as homologous. In some Palaeoniscids the hyomandibula is, however, pierced by a canal for at least a branch of the truncus hyomandibularis VII. The foramen is placed differently in different genera. So it lies near the anterior border of the hyomandibula in *Broughia* or near the posterior border for instance in *Glaucolepis*, where it pierces dorsally to the basis of the opercular process. In *Saurichthys* there is a long groove on the lateral surface of the hyomandibula, indicating that a branch of the hyomandibular nerve ran in immediate contact with it. Regarding the general agreement between the hyomandibula of the Palaeoniscids and that of *Polypterus* it seems most likely that the hyoid branch of the truncus hyomandibularis ran behind, the mandibular branch in front of the hyomandibula. The hyoid branch probably pierced the hyomandibula caudally at least when the foramen was located near its posterior border. Whether the foramen for instance of *Broughia* contained the mandibular branch or the hyoid branch or the undivided truncus hyomandibularis, is impossible to decide. The general agreement of the hyomandibula of *Polypterus* with that of the Palaeoniscids makes it most probable (compare STENSIÖ, 1925, 1932 and NIELSEN, 1942) that the truncus hyomandibularis divided already medially to the hyomandibula and that its two chief branches went on each side of the skeletal piece and close to it. Then the enclosing perhaps took place through lamellae of the perichondrial bone overgrowing the nerve branch, in a manner corresponding to that common in bony fishes (where, however, also the hyomandibular cartilage is generally pierced). According to ALDINGER (1937) there is in *Pygopterus* and *Acropholis* a shallow groove at the posterior border of the hyomandibula, in which the hyoid nerve probably ran. In *Boreosomus* this groove is covered by a thin bone lamella so that the groove is enclosed to a foramen. This statement seems to give evidence for the preceding hypothesis.

In some Palaeoniscids a symplecticum and a stylohyal have been found at the lower end of the hyomandibula (STENSIÖ, 1932, NIELSEN, 1942). In *Broughia* STENSIÖ (1932) found a short pointed symplecticum attached to the antero-ventral corner of the hyomandibula in a position somewhat answering to the symplectic process of *Polypterus* described in preceding lines. He also found a rather weak stylohyal at the lower end of the hyomandibula. NIELSEN (1942) described a symplecticum in species of *Glaucolepis* and in *Boreosomus*, but no stylohyal. In *Glaucolepis* he found two centres of ossification in the ceratohyal. Is it then not possible that the posterior centre might represent the stylohyal

VOL. 24 1943

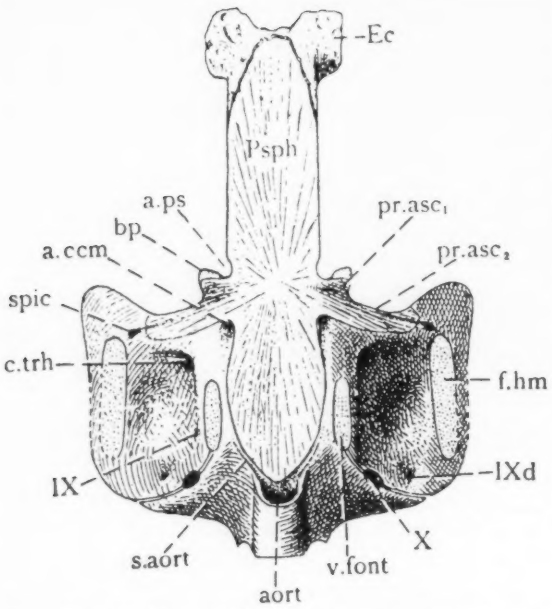


Fig. 46. *Broughia perleididoides* STENSIÖ. Ventral view of the neuro-cranium (from STENSIÖ, 1932).

(ALLIS' epihyal in *Polypterus*)? in *Polypterus* the stylohyal is about as strong as the ceratohyal but much shorter than the posterior ossification in *Glaucolepis aldingeri*.

Disregarding the behaviour of the hyomandibular nerve the hyomandibula of *Polypterus* and of the Palaeoniscids is built as that (of bony Ganoids and) of Teleosts, where its development has been investigated in preceding pages. From these descriptions it is clear that the hyomandibula of these latter fishes is composed of a pharyngo- and an epihyal to which is added a ray-bar posteriorly. This bar forms dorsally the posterior articular head of the hyomandibula and ventrally the opercular process. Fronto-ventrally it has a symplectic process and postero-ventrally it articulates with a stylohyal. This description coincides perfectly with that of the hyomandibula of *Polypterus*, where, however, the pharyngeal element is not discernible as a separate part. But in adult ganoids and teleosts this part also

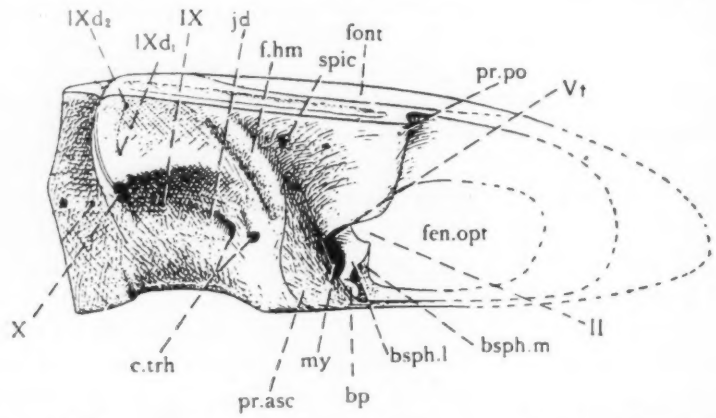


Fig. 47. *Perleidus stoschiensis* STENSIÖ. Lateral view of the endocranium (from STENSIÖ, 1932).

is not delimited at all, only discernible in early embryos. When the hyomandibula of all these fishes is quite comparable, the conclusion must be that the position of the hyomandibular nerve and its branches is variable. It is the nerve that is responsible for its different relations to the hyomandibula and not the hyomandibula as has supposed ALLIS (1915). The hyomandibula of the recent Chondrostei (*Acipenser* and *Polydon*) differs from that of the other ganoids in that there is no posterior articular head present and the supposed opercular process is rudimentary and placed on the lateral side of the epihyal.

The articular fossa for the dorsal end of the hyomandibula is oblong and placed obliquely in *Polypterus*. Its anterior border is situated on a level be-

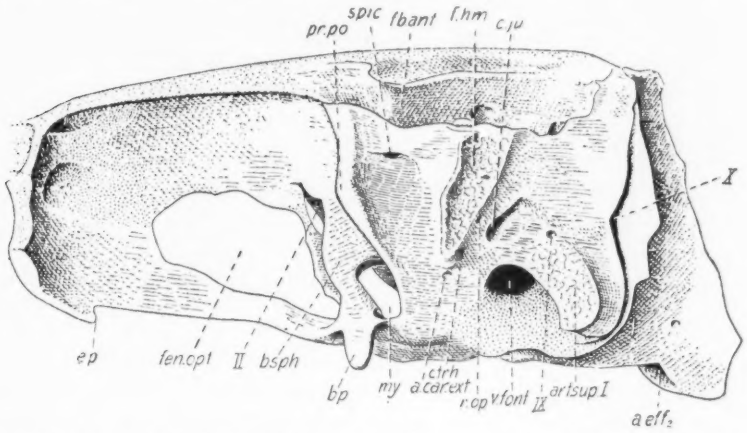


Fig. 48. *Glaucolepis Stensiöi* NIELSEN. Lateral view of the endocranium (from NIELSEN, 1942).

hind the posterior opening of the jugular canal at a distance dorsally to the opening. Its posterior border is dorsally on a level with the so-called parietal bone. In the Palaeoniscids the hyomandibular facet has a somewhat different position in different genera. Mostly the facet is oblong, frontally narrower than caudally and located obliquely with the anterior border, lower than the posterior. In *Broughia* (STENSIÖ, 1932) it is, however, almost horizontal (fig. 46). The anterior border of the facet lies mostly on a level dorsally to the jugulo-hyomandibular canal, as for instance in *Ospia, Boreosomus* and *Saurichthys*. In *Perleidus* the anterior border of the facet extends so far ventrad that it comes in front of the foramen of the jugulo-hyomandibular canal and probably occupies a dorsal part of the lateral commissure (fig. 47). In *Glaucolepis,* where the facet is almost vertical, its lower end lies on a level ventrally to the jugal canal (NIELSEN, 1942), and at least its lower half is situated upon the lateral commissure (lateral wall of the trigemino-facialis chamber) (fig. 48). (This is a statement which will be used further on p. 103).

g) *The hyomandibula of the Crossopterygians.*

The hyomandibula of "*Megalichthys*" *nitidus* has been fully described by ROMER (1941). From this description it is clear that this element is built up in full agreement with that of a teleostomian and therefore must be considered to be composed similarly viz, of a pharyngo- and an epihyal to the

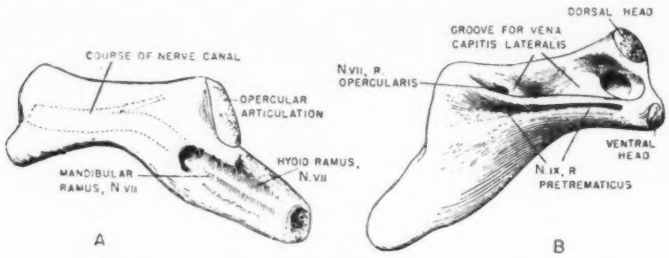


Fig. 49. "*Megalichthys*" *nitidus*. Hyomandibula A) lateral, B) medial aspect (from ROMER, 1941).

posterior border of which is plastered a ray bar of which the upper part forms the upper (posterior) articular head and the lower part the opercular process. See further p. 102 (fig. 49).

h) *Summary concerning the hyomandibula.*

In the preceding pages I have assembled a body of material sufficiently to allow some conclusions to be drawn regarding the hyomandibular problems. It consists of embryological, comparative anatomical and paleontological statements concerning the hyomandibula proper and its relations to blood-vessels, nerves and muscles, partly at different stages of development. The most important of these statements will be briefly enumerated below.

Fig. 50. "*Megalichthys*" *nitidus*. Position of the hyomandibula (from ROMER, 1941).

1) The early rudiment of the hyomandibula (epihyal part) is always located immediately behind the spiracular canal; its rudiment lying close to the lining epithelium of the branchial intestine.

2) At these early stages the head-vein always lies dorsally to the epihyal rudiment, either dorso-laterally, quite dorsally or dorso-medially to it. At earlier stages the vein lies more laterally than at later stages.

3) To the epihyal element there is always added a pharyngohyal, which either fuses on to the epihyal (sharks and teleostomians[1]) or to the auditory capsule (rays and *Acipenser*).

[1] When speaking of Teleostomians the *Dipnoi* are excluded in the following pages.

4) In Teleostomians there is always in early embryos an inconsiderable medial process on the upper end of the epihyal. This process represents an infrapharyngohyal. (It always disappears as a process during ontogeny.)

5) In Teleostomians the pharyngohyal is a suprapharyngohyal. It always forms (so far as is known) dorsolaterally to the upper end of the epihyal, i.e. in a more superficial position than the epihyal. Owing to this position it gains contact with the auditory capsule laterally (dorsally) to the head-vein.

6) In Teleostomians there is always a ray-bar plastered on to the pharyngo-epihyal.

7) The principal head-veins are the vena capitis medialis (in early embryos), the lateralis and the secondary vein.

8) In Selachians the principal head-vein is the *vena capitis lateralis.*

9) In ganoids and teleosts the principal head-vein is the *secondary vein,* the vena capitis lateralis only being present dorsally to the glossopharyngeal nerve. (Compare pp. 127—140.)

10) In Selachians the hyomandibula is located inside, i.e. ventrally to the head-vein, in teleostomians outside, i.e. dorsally to it.

11) The hyomandibular nerve (truncus hyomandibularis) always runs out in front of the hyomandibula in embryos.

12) In sharks and rays the hyomandibular nerve runs perfectly in front of the hyomandibula.

13) In Chondrostei the proximal part of the hyomandibular nerve runs inside the hyomandibula and then crosses it on the outside.

14) In *Polypterus* the hyomandibular nerve branches inside the hyomandibula, then sending out the mandibular branch frontally, the hyoid branch caudally to the hyomandibula; so it was probably the case also in Palaeoniscids.

15) In Crossopterygians, bony Ganoids and Teleosts the hyomandibular nerve enters the hyomandibula on the inside and runs through a canal, leaving it on the outside (figs. 49, 50).

16) In sharks the hyomandibula consists of the epihyal and the pharyngohyal fused together.

17) In *Acipenser* the hyomandibula consists of an epihyal, a suprapharyngohyal and an opercular process, probably developed from a hyoid ray-bar plastered on the epihyal on its outside. The hyomandibula has but one articular head (the anterior).

18) In bony Ganoids and in Teleosts the hyomandibula has two articular heads and consists of an epihyal with a symplectic process fronto-ventrally, a suprapharyngohyal dorsally and a ray bar, consisting of a dorsal ray forming a posterior articular head and a ventral portion with the opercular process. In *Lepidosteus* in addition a subopercular ray (subopercular process) is present.

VOL. 24 1943

19) In Palaeoniscids the hyomandibula is as in living bony ganoids and teleosts. A pharyngohyal, however, cannot be demonstrated as it might have been present only in the embryo.

20) In Crossopterygians the hyomandibula is probably the same as in Palaeoniscids, thus consisting of an epihyal (+ pharyngohyal?) and a ray bar on its caudal border. It is certainly the same as in ganoids and teleosts, but without the symplectic process, the symplectic being separate.

21) In *Polypterus* the hyomandibula is a hyosymplectic with an inconsiderable symplectic part. It is else built as in Palaeoniscids.

22) In sharks and rays the hyomandibular facet lies below the head-vein, in Ganoids and Teleosts it lies dorsally to it, but in the Crossopterygians it has an articular head ventrally and another head dorsally to the head-vein. In *Rhizodopsis* (SÄVE-SÖDERBERGH, 1936) the dorsal articular facet is very long, vertically located on a level with the posterior border of the lateral commissure and partly extended over the upper end of the commissure: In "*Megalichthys*" *nitidus* both facets are more or less circular and rather deep (ROMER, 1937) and both facets seem to be located at a level posteriorly to the lateral commissure (ROMER, 1941).

23) In living Ganoids and Teleosts and in some Palaeoniscids the hyomandibula is placed more or less vertically to the longitudinal axis of the endocranium, in the Crossopterygians and in most Palaeoniscids more longitudially.

THE THEORY OF SCHMALHAUSEN AND OTHERS CONCERNING THE DIFFERENT POSITION OF THE HYOMANDIBULA IN SELACHIANS AND TELEOSTOMIANS.

It is a wellknown fact that the articular facet for the hyomandibula is located below the head-vein in Selachians, above it in Teleostomians. Many attempts have been made to explain this striking difference. The most acceptable of them is the theory of SCHMALHAUSEN, DE BEER, SÄVE-SÖDERBERGH and ROMER. According to these authors the original hyomandibula has two heads, one on each side of the head-vein. In the Selachians the ventral head, in the Ganoids and Teleosts the dorsal one was retained, the other being reduced. Since SÄVE-SÖDERBERGH and ROMER have stated that this —originally merely postulated—condition was actually present in the Crossopterygians, the theory has acquired very strong support. Nevertheless I do not consider it to be satisfactory for the following reasons. The only way in which we can expect to arrive at an acceptable interpretation of the hyomandibula in Crossopterygians is by way of comparison with those living fishes the organisation of whose hyomandibula is known: with that of living

ganoids and teleosts. The external features of the hyomandibula, of ganoids and teleosts are very similar to those in the Crossopterygians. In both cases there are two articular heads, an opercular process and a canal for the hyomandibular nerve. The only difference seems to be that the hyomandibular nerve branches already in the interior of the hyomandibula in the Crossopterygians, but after leaving it in the Teleosts. The measure of agreement between the Crossopterygian and teleostean (ganoidean, palaeoniscidean) hyomandibula is so great that it is impossible to assume that they are not organized according to the same scheme. (Compare preceding pages.) In *Salmo* the composition of the hyomandibula was explained in the preceding pages. It consists of an epihyal, to which is added a suprapharyngohyal and

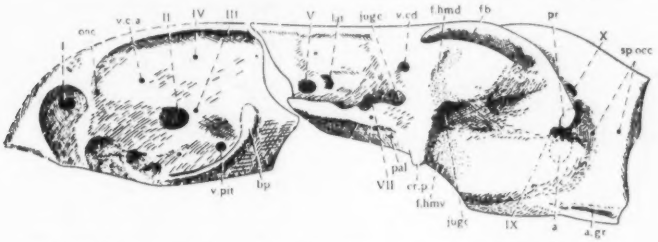


Fig. 51. *Rhizodopsis sauroides.* Lateral view of the endocranium (from SÄVE-SÖDERBERGH, 1936).

a branchial ray bar. The posterior articular head consists of a ray fused on to the rest of the hyomandibula. The conclusion from these statements must be that the upper articulation of the crossopterygian hyomandibula is between a hyomandibular ray and the neural skull, thus secondary. This ray has gained support on the neurocranium dorsally to the head-vein, the lower articulation being the primary, comparable with that in Selachians. If these conclusions are correct it remains for us to find an interpretation of the different locations of the teleostean and the crossopterygian hyomandibula in relation to the head-vein. The location of the articular facets on the neurocranium of the Crossopterygians indicates a possible means of shifting the crossopterygian hyomandibula to a quite dorsal location. In *Rhizodopsis* (fig. 51) and *Ectosteorhachis* ("*Megalichthys*") (fig. 52) the dorsal facet partly lies upon the lateral commissure and thus in front of the posterior opening of the canal for the head-vein. In *Osteolepis* WATSON (1925) found and figured an articular facet situated *upon* the lateral commissure in front of the posterior opening of the jugular canal. Thus the caudal part of the lateral comissure seems to be an easy means of shifting the entire hyomandibula from ventral to dorsal position without disturbing other structures.

In the Palaeoniscids the position of the hyomandibular facet also is suggestive of a shifting. In *Boreosomus* (fig. 48) the anterior (ventral) head lies almost completely upon the lateral commissure, in *Perleidus* (fig. 47) the

VOL. 24 1943

anterior head lies somewhat more dorsally, in most genera it is fully dorsal in relation to the lateral commissure. In these cases the facet is oblique. In *Broughia* (fig. 46) at last the facet is horizontal as in living ganoids and bony fishes.

I think that these are sufficient reasons for assuming a shifting of the anterior articular head by way of the lateral commissure from ventrally to the head vein to dorsally to it. This conclusion agrees well with the ideas set forth by STENSIÖ in his paper of 1925, p. 145.

Supposing that the teleostomian condition can be derived from selachian, one can interpret the evolution of the former as follows.

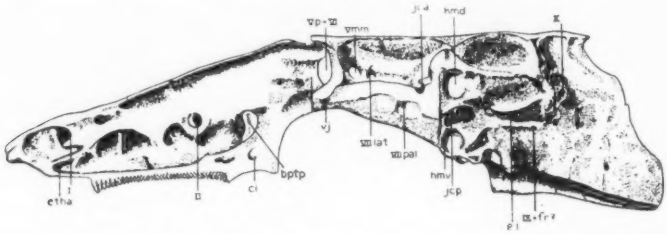


Fig. 52. "*Megalichthys*" *nitidus*. Lateral view of the endocranium (from ROMER, 1937).

1) In sharks the hyomandibula is a pharyngo-epihyal. On its posterior margin a ray bar with separate rays is present. The hyomandibula articulates with the endocranium behind the lateral commissure and lies ventrally to the head vein.

2) In a precrossopterygian stage the uppermost ray of the ray-bar attained contact with the neurocranium dorsally to the head vein. Another ray developed into an opercular process.

3) In the crossopterygian stage the supporting ray fused on to the rest of the hyomandibula, which thus became its two articular heads, one on each side of the head vein.

4) The two articular heads shifted frontad to a level with the lateral commissure.

5) By means of the lateral commissure the two heads shifted dorsad to attain at last a quite dorsal and horizontal position.

THE STYLOHYAL PROBLEM.

The hyoid arch in sharks consists of three parts, viz. the hyomandibula (pharyngohyal+epihyal), the ceratohyal and the hypohyal. In *Polypterus*, Ganoids and Teleosts there are four, viz. the hyomandibula, the stylohyal (or interhyal)[1], the ceratohyal and the hypohyal, whereas in the branchial

[1] Epihyal according to ALLIS, 1922.

arches there is no portion corresponding to the stylohyal. This difference between Selachians and Teleostomians involves a problem of considerable interest which was explained by assuming that a new segmentation of the hyoid arch had taken place in Teleostomians (SEWERTZOFF, 1928). It has been said that the stylohyal segments off from the upper end of the ceratohyal (SEWERTZOFF, STÖHR (1882)).

The preceding description of the development of the hyoid arch in *Salmo salar* seems to throw some light upon the stylohyal problem. It was stated that the stylohyal rudiment belongs to a layer of skeletal tissue that is different from and more superficial than that to which the hyo-symplectic and the ceratohyal belong and that it is attached to the lateral surface of the rudiment of the hyal arch proper as a lateral outgrowth of some independence. The mode of its development led to the conclusion that in *Salmo* the stylohyal may correspond fairly closely to a branchial ray belonging to the hyal arch, a ray developed in connection with the future ligamentous connection between the hyo-symplectic and the ceratohyal. Such an interpretation is, however, far from certain and it will probably never be certain considering the scarcity of data, and this always precludes any certainty in questions of this nature. The theory, however, might perhaps gain support if it could be demonstrated that similar conditions are present in other teleostomian groups possesing a stylohyal, viz. in *Acipenser, Lepidosteus* and *Amia.* I have therefore undertaken to investigate those fishes more closely. My material, however, is at many points insufficient for a complete description of the development of the stylohyal; nevertheless it has given some remarkable results, which are summarized in the following lines.

Acipenser. The development of the hyoid arch in *Acipenser ruthenus* has been described at some length by SEWERTZOFF (1929). At the earliest stage (neither the body length nor the age of the embryo are given) at which the hyoid arch was studied by him it consisted of a dense mesenchymatic rod slightly S-shaped. It does not reach the auditory capsule and the hyomandibular nerve runs dorsally to it. At a later stage the hyoid arch has grown considerably in length, and chondrification has begun in its hyomandibular and ceratohyal parts, whereas its middle part consists of younger tissue. This region forms the (upper) frontad curve and corresponds to the symplecticum + stylohyal. The upper end of the arch reaches the lower surface of the otic capsule. At a more advanced stage (SEWERTZOFF, Pl. VI, fig. 25) the S-curves of the arch are sharper than before. At the lower curve, with frontal convexity, there lies the rudiment of the symplectic process, not yet process-like, and behind and below it the rudiment of the stylohyal. All the parts of the hyoid arch together form a continuum, but the future lines of demarcation can nevertheless be determined by differences in the histological structure and disposition of the prochondrial tissue. At a yet later stage

VOL. 24 1943

the arch has become segmented in the future parts, viz. the hyomandibula, the symplectic, the stylohyal, the ceratohyal and the hypohyal. The difference between the segmentation of the hyoid arch in selachians and teleostomians is said to depend upon a new segmentation of the arch in the latter, whereby it has obtained 5 (6) elements instead of 4 (5) in the former.

In *Acipenser Gyldenstaedti, ruthenus* and *stellatus* the development of the stylohyal is achieved in a corresponding manner. I shall here describe it in *Gyldenstaedti.* In this sturgeon the rudiment of the stylohyal is to a certain extent independent of the rest of the hyoid arch as it develops into a more superficial layer than the arch proper. As the epihyal part becomes differentiated from the ceratohyal there remains a broad blastematic band between these two parts, on the outside of which the stylohyal rudiment is located. The stylohyal subsequently chondrifies and the chondrification extends to the band on its inside, which also chondrifies in continuity with the primary stylohyal rudiment. The definitive stylohyal thus consists of one part belonging to the hyoid arch proper and of another corresponding probably to a hyoid ray, as was assumed to be the case with the primary stylohyal in *Salmo salar* (p. 93).

Lepidosteus platystomus: The development of the hyal arch of *Lepidosteus platystomus* has been described by HAMMARBERG (1937). The description was based on the material of the Zootomical Institute. The hyal arch at the 11 mm stage forms a continuum from the auditory capsule dorsally to the ventral side of the head. In this arch, however, different portions are discernible owing to the different stages of chondrification. Thus a median (medium) blastematic portion, including the rudiment of the symplectic process and the stylohyal, separates the prochondrial hyomandibula from the ceratohyal. At the 12 mm stages all elements of the hyal arch are well delimited but form a continuous cartilage bar. At the 16 mm stage the different parts of the hyal arch also form a cartilaginous continuum. At the 18.7 mm stage the stylohyal has become separated from the hyomandibula as well as from the ceratohyal and articulates against these parts.

I have investigated the same material as HAMMARBERG, and I am able to confirm all his results, though concerning the stylohyal there is an important detail to be added. At the 11 mm stage the hyomandibular portion is broadly connected with the ceratohyal by a blastema lying close to the lining epithelium of the buccal cavity. The lateral portion of this blastema contains a rather well delimited lens of prochondrium. At the 12 and 12.3 mm stages the connecting portion as well as the lens are chondrified. The same is the case at the 16 mm stage. At the 18.7 mm stage the stylohyal has become a separate element. According to its development it thus contains a medial and a lateral portion completely fused on to one another.

Amia calva. At an early stage of development the hyal arch forms a continuous band of mesenchyme lying close to the lining epithelium of the buccal cavity. Later, as the rudiment of the stylohyal appears, the primary connection between the future hyomandibula and the ceratohyal has acquired a bend caudad and the rudiment of the stylohyal frontolaterally borders this bent portion of the arch. At such a stage it is only possible to delimit the rudiment from the primary arch by its lateral position. Later, as prochondrium appears, a limit is clearly discernible since prochondrium develops first of all in the stylohyal rudiment, whereas the primary blastema remains as a blastematic band. At this stage the stylohyal bends out from the epithelium of the buccal cavity, but the blastematic connection between the hyomandibula and the ceratohyal remains in its primary location. The ceratohyal of this stage has already got a clublike caudal head. The connecting band is connected with the mesial portion of the articular head of the ceratohyal and extends upwards to the mesial part of the hyomandibula. The stylohyal rudiment now forms a more lateral connection between the hyomandibula and the ceratohyal, being prochondrially continuous with the lateral portion of the hyomandibula dorsally and blastematically connected with the lateral portion of the ceratohyal head ventrally. This condition seems to indicate that if the stylohyal is a branchial ray, it may be a ray belonging to the hyomandibula.

VOL. 24 1943

At a later stage of development chondrification has taken place and the primary stylohyal has chondrified together with the connecting band. The double origin of the resultant cartilage, however, is clearly demonstrated in cross-sections by two circular centres of chondrification. The (secondary) stylohyal has now become articulated against the hyomandibula as well as against the ceratohyal.

The development of the stylohyal of *Polypterus* is still unknown.

1) In *Acipenser, Lepidosteus, Amia* and *Salmo* the definitive stylohyal consists of two parts, a medial and a lateral. The medial portion is part of the primary hyoid arch, the lateral during development is added to the former and may be explained as a branchial ray belonging to the epihyal portion of the hyomandibula.

2) The relative primary independence of the lateral part (ray element) of the stylohyal seems after chondrification to cause the segmenting-off of the medial (also chondrifying) portion from the epihyal and the ceratohyal respectively.

THE SYMPLECTICUM.

The presence of a symplectic has always been considered to be a special character belonging to teleostomian fishes. ALLIS (1915) says that if the

symplectic of the telostei is not simply a ventral process of the hyomandibula "it must be looked for in other cartilages of the region, and it would seem as if it must be a specially modified ray of the mandibular arch. That it is an entirely new element here added to the hyal arch seems wholly improbable". ALLIS gives good reasons for the theory that the symplectic is a modified mandibular ray. He points out that according to STÖHR (1882) the symplectic is developed as a primarily independent element which lies close to the hind edge of the palato-quadrate and later fuses with the hyomandibula. (In my material of *Salmo salar* I cannot affirm STÖHR's statement, as the symplectic there always is a process of the hyomandibular blastema.) He also emphazises that the symplectic lies inside the afferent mandibular (pseudobranchial) artery, which is the relation of a branchial ray of the mandibular arch. Furthermore, the symplectic is frequently fused on to the hind end of the palato-quadrate, which is natural for a branchial ray of that cartilage. To these reasons are to be added its independent chondrification in *Acipenser* and independent ossification in bony Ganoids and Teleosts and its independence in Palaeoniscids and Coelacanthids.

In the first and third parts of this work (HOLMGREN, 1940, 1942) I have shown that the mandibular arch and the hyomandibula in sharks and rays are united by an early separate element which undoubtedly must be a mandibular arch ray, independent especially in early embryos of *Urolophus* and *Raja* but also partly in *Torpedo* and in sharks. It has there the characteristic relations of a branchial ray. In older embryos this ray fuses on to the hyomandibula and disappears as a separate element. In *Potamotrygon* and *Mobula* there are two such rays present which persist in the adult as separate cartilages (GARMAN, 1913, HOLMGREN, 1942).

The symplectic thus is no special character of teleostomians only, but is already present in selachians.

THE RELATION OF THE MANDIBULAR ARCH TO THE HYOMANDIBULAR AND TO THE NEUROCRANIUM, PHYLOGENETICALLY CONSIDERED.

In gnathostomian fishes the following connections between the palato-quadrate and the neurocranium are present:

1. The orbital connection between the orbital process of the palato-quadrate and the trabecula in sharks (but not in rays). As demonstrated in the preceding pages of this paper, this connection is probably homologous with the posterior ethmoid connection in Teleosts and bony Ganoids and the orbital (palatobasal) connection in *Acipenser*.

2. The basal or basitrabecular (basipolar) connection, rudimentary in sharks, in *Acipenser* and in *Lepidosteus* (HAMMARBERG, 1937) and probably also in Teleosts. In rhipidistid Crossopterygians, devonian Coelacanthids and Palaeoniscids it was a functional articulation (In Chimaeroids and Dipnoi the connection is cartilaginous without articulation).

3. The lateral commissure or processus oticus internus, a part of the mandibular ray bar fused at its dorsal and ventral ends on to the neurocranium laterally to the jugular vein and the hyomandibular nerve. A lateral commissure is present in Selachians, Crossopterygians, Palaeoniscids, *Chondrostei, Holostei* and Teleosts. In *Dipnoi* the processus oticus internus is fused on to the neurocranium at its upper end. A lateral commissure is primary absent in *Acanthodii, Arthrodira* and secondary in some *Batoidei* and many teleosts.

4. The processus oticus externus, which in some Selachians and Acanthodians forms an articulation with the postorbital process, externally to the lateral commissure, when simultaneously present. The processus oticus externus is in most cases more or less rudimentary, not reaching the postorbital process.

5. An articulation between the palato-quadrate and the "antotic" process in Caelacanthids. In Rhipidistids this articulation seems to have been absent. The articular process of the Caelacanthid palato-quadrate must be an ascending process, with the epipterygoid ("metapterygoid") bone. Behind this process another process probably represented the processus oticus externus (STENSIÖ, 1932).

6. A secondary articulation between the palato-quadrate and the lateral commissure in *Lepidosteus* (HAMMARBERG) (described in literature as the basipterygial or basal process).

7. A connection between the posterior end of the palato-quadrate and the otical shelf (mandibular ray?) in embryonic sharks and in Chimaeroids (chondrified) (HOLMGREN, 1942).

8. A longitudinal membranous connection between the upper border of the palato-quadrate and the trabecula in sharks and rays and teleostomians. In *Scyllium* this membrane chondrifies medially and the chondrified part (subocular cartilage) fuses on to the trabecula. In Chimaeroids the corresponding connection is cartilaginous throughout.

9. The processus ascendens in Dipnoi (and Amphibians). Its occurence in Teleostomians is otherwise problematic. In Crossopterygians the "epipterygoid" probably corresponds to the ascending process. (Compare sub. 5.)

10. The relation of the symplectic to the mandibular arch is variable. In sharks and rays the symplectic which partly forms the lower end of the hyomandibula (HOLMGREN, 1940), is connected with the posterior end of the mandible. In *Acipenser, Polyodon* and at least *Boreosomus* and *Glaucolepis* among the Palaeoniscids the symplectic is articulated against the palato-

VOL. 24 1943

quadrate as well as aganist the lower yaw. In bony Ganoids (and *Polypterus*) and Teleosts the symplectic is chiefly connected with the palato-quadrate, the processus retroarticularis of the maeckelian cartilage being connected (not in *Polypterus*) with the hyal arch by means of a strong ligament, the ligamentum mandibulo-hyoideum. In *Latimeria* (SMITH, 1940), the recent Coelacanthid, this process is connected with the hyomandibula by a long, narrow "symplectic". This "symplectic" probably is comparable with the said ligament. Elsewhere in this paper I have joined ALLIS opinion of the symplectic as a ray belonging to the mandibular arch, and pointed out, that two such rays are present in *Potamotrygon* and *Mobula*, both articulated against the lower yaw The possibility thus seems not to be excluded that there are different symplectic, i.e. consisting of rays belonging to the palato-quadrate and to the mandible respectively.

11. In Teleosts and in Holostei the palatine part of the palato-quadrate is fused on to the lower side of the ethmoid plate (see p. 40). In the chondrostei the mesenchymatic part of the barbels may possibly represent the palatine part. It is not possible to tell whether a palatin part of the palato-quadrate was ontogenetically present in Palaeoniscids and Crossopterygians. In the adult no such part has been recognized.

12. The metapterygoid bone of *Amia* and of the Teleosts agrees so much with the metapterygoid bone in Coelacanthids, that the homology seems probable. The pedicle process of *Amia* thus may be the processus ascendens and the process behind (in the embryo) the metapterygoid the processus oticus externus. (Compare ALLIS.)

13. In myxinoid Cyclostomes the palato-quadrate is present only in embryos, but a mandibular apparatus newertheless is well developed, formed by three bars of cartilage: a mandibular, a postmandibular (with an unpaired postsymphysial plate) and a ponticular (HOLMGREN, 1942). These parts seem to have been present in the *Antiarchi* and probably also in the Chimaeroids and perhaps also in the Acanthodians (HOLMGREN, 1942). Are the interpretations of the mandibular apparatus of these fishes correct, so becomes the question of the phylogenetical initial stage of the gnathostome yaws much more complicated than without knowledge of the mentioned conditions. Is it true that the yaw apparatus of the *Antiarchi* can be explained on the basis of the lower yaw complex of *Myxine* and that the conditions in the Chimaeroids are explained by those in the *Antiarchi*, it seems necessary to conclude, that the simple lower yaw in the other fishes has arisen by means of a reduction of the more complicated. (The presence in *Narke* (an electric ray) of a cartilage probably belonging to one of the two posterior series of the yaw cartilages in the *Antiarchi* is suggestive for the acceptance of such a reduction). Starting from this standpoint it seems reasonable to believe, that a yaw mechanismus more or less as that in *Myxine* was characteristical to

the predecessors of the Gnathostomians, the more as the conditions in *Petromyzon* (which is not nearly related to *Myxine*) are derivable from those in *Myxine* (HOLMGREN, 1942). In *Myxine* the lower yaw complex is combined with the great tooth-plates of the mouth cavity. Is it then too hazardous to refer the explanation of the gnathostomian conditions to such a mechanismus, as that of Myxinoids in connection with tooth-plates of some description, either horny or ossified? The complexity of the mandibular cartilage then was possibly an adaption for carrying the plates and was perhaps preceded by a simplier apparatus or a single maeckelian cartilage. Is this interpretation correct, a kind of *palaeostylic* condition (GREGORY, 1904, DE BEER, 1931), if ever present, must have preceded the complicated cyclostomian—antiarchian—holocephalian, and not that of other gnathostomian fishes, which developed from the more complicated conditions as those in the latter through reduction of the posterior parts of the apparatus.

VOL. 24 1943

What are the two posterior bars, assuming that the anterior represents the maeckelian cartilage? According to the branchial arch theory of the mandibular arch, the explanation of the two posterior bars in *Myxine* must be that they represent the mandibular ray bar, divided in two different bars. Such a differentiation of a ray bar is no rare occurence in fishes, for instance in rays where the pseudohyoid is the anterior part of the ray-bar, where the hyoid rays represent the posterior part. In *Chimaera* the rays of the hyal arch also are fixed on cartilaginous plates, probably formed by fusion of basal parts of the rays. In many sharks similar conditions are present. Also in *Polypterus* the rays issue from a continuous basal cartilaginous band. In this connection it may be pointed out that there are two such bars in most Selachians, the proximal forming the lateral commissure, the distal the spiracular cartilage or cartilages. The difference is, however, that there are no rays developed in *Myxine,* but the "rays" may nevertheless be represented by the posterior bar, the ray bases by the anterior.

As the palato-quadrate of *Myxine* is only represented as a mesenchymatic rudiment in the embryo and in *Petromyzon* as yet is not clearly defined, it is impossible to tell how it was organized in such "agnathous" fishes which had it fully developed. Perhaps it was (at least ontogenetically) differentiated as in a gnathostome fish.

14. In *Myxine*—the only "agnathous" fish where a true branchial arch has been demonstrated—this arch is undivided. So was also probably the case in the Cephalaspids, so marvellously studied by STENSIÖ. This condition was probably correlated to the fact that the gills are or were enclosed in branchial sacs with narrow external openings or with a common external opening. Then there arises the question, is there not left any trace of the marsipobranch condition in the branchial arrangement of the gnathostomians? In such a case the only structure which possibly could represent such a remainder from the

marsipobranch condition, is the spiracular canal and gill. The occurence of a spiracle undoubtedly gives some support for the assumption that the spiracle is a structure of very great age, as it is or was present not only in all gnathostomian fish groups, beginning with the *Antiarchi* and Arthrodires. In my paper of 1942 I have given reasons for considering the *Antiarchi* as pregnathostomians related to "agnathous" fishes such as the Cephalaspids and Myxinoids. Under such circumstances it would not be surprising if a marsipobranch condition remained in the gnathostomians. Against this hypothesis speaks, however, the generally accepted opinion, that the spiracle of the selachians developes through closing from below of a perfect gill-slit of the embryo. This opinion which I have also earlier accepted (HOLMGREN, 1942), is not well founded. The foremost gill-slit of for instance a *Squalus* embryo is a rather long slit and but a little shorter than the following slits. The foremost gill-slit lies, however, dorsally to the future lower yaw articulation and does not extend behind the mandible (ceratomandibular). The branchial gillslits extend ventrally behind the ceratobranchials. The foremost gillslit (the mandibular, or spiracular) thus never comprises more than the dorsal half of a perfect gill-slit. This half then closes from below to form the spiracle. The contradiction between an hypothetical phylogenetical remainder of the marsipobranch branchial typus and the ontogenetical development of the spiracle thus is only apparent. This statement involves the necessity of revision of our interpretation (HOLMGREN, 1942) of the way in which the hyomandibular became suspensorial. If only the dorsal part of the mandibular gillslit was developed, it is obvious that a junction of the mandibular skeletal arch and the hyoid arch could take place without closing of any gill-slit, thus immediately below the spiracle, where the mandibular and hyoid mesenchymes always are continous.

As far as the scanty facts reach the visceral arches of the "agnathous" fishes were undivided, excepting the mandibular. In the gnathostomians the visceral arches are all divided in their elementary pieces. It seems reasonable to believe, that this division took place in correlation to the extending of the narrow branchial openings to long transversal gill-slits with mobility in different directions (frontal, lateral and dorsal). The subdivision of the branchial arches thus probably was acquired by the gnathostomians. I therefore assume two different ancestral types of the mandibular apparatus: an *archistylic* in the *Agnatha* and an *palaeostylic* in the early Gnathostomians. The latter must have developed from the former.

The question now is to marshal the mentioned facts and considerations in such order as will enable us to establish an acceptable phylogenetical series.

I. *Archistylic type.* Hypothetical. With undivided visceral arches and marsipobranch type of the branchial apparatus, with narrow branchial openings.

I a. *Myxinoid type.* The mandibular arch was divided in a dorsal palato-quadrate and a ventral mandible, the latter with two (branchial) ray bars, developed as cartilaginous rods. As the palato-quadrate is only present as such in the embryo and then almost completely disappears, it is impossible to tell whether it was subdivided or not. The hyoid and the first branchial arch are undivided.

I b. *Petromyzon type.* No inner branchial arches. The mandibular arch contained a mandible built according to the same type as that in Myxinoids. The existence of a palato-quadrate is doubtful.

II. *Palaeostylic type.* Hypothetical. (GREGORY, 1914, DE BEER, 1931). As our interpretation of the mandibular arch of a gnathostomian fish is based upon the morphology of a normal branchial arch, a primitive gnathostomian form of a mandibular arch was considered to have been formed as a normal branchial arch with the supra- and infra-pharyngo-mandibulars as the only suspending processes and with branchial rays developed on the epimandibular at least. Between the mandibular and hyoid arches there must have been present a gill-slit, which separated the dorsal parts of the two arches. This hypothetical initial stage of jaw suspension coincides well with the so-called Palaeostylic (GREGORY, 1914, DE BEER, 1931) suspension, but with the difference that two pharyngomandibulars operate this suspension instead of only one. The lower yaw consisted of three bars of cartilage, the mandibular, the postmandibular and the ponticular.

The palaeostylic suspension may have developed from a primitive archistylic, but how this development took place cannot be demonstrated. Perhaps the transformation of the narrow gill-openings of a marsipobranche type into a slit-like of a gnathostome type worked the segmentation,—as already explained (p. 112)—,of the originally undivided branchial arches into its gnathostomian pieces.

III. (*Autodiastylic,* DE BEER, 1933). At a following (hypothetical) stage of development the posterior end of the epimandibular part of the arch swung dorsad so that it became more or less parallel with the trabecula. The infrapharyngobranchial was thereby directed frontad and developed into the palatine part of the palato-quadrate. The suprapharyngobranchial retained its articular connection with the trabecula and became the orbital process. A longitudinal membranous connection developed between the epimandibular and the trabecula and the polar cartilage. The altered position of the epimandibular involved a transformation (extra-palato-quadrate) or disappearance of the upper (anterior) epimandibular rays, whereas the lower (posterior) retained their character of branchial (spiracular) rays. At this stage a primitive spiracle probably was present or arose. The hyoid arch was not suspensorial, but it was probably already connected with the mandibular arch through ligaments (or muscles), running ventrally to the spiracle. This hypo-

VOL. 24 1943

thetical stage with orbital suspension (orbitostylic) seems to have been realised in some Acanthodians (*Climatius, Mesacanthus*). In other types such as *Acanthodes* the extra-palato-quadrate was developed into a processus oticus externus with suspensory function (*basiotostylic* condition (DE BEER, 1931)). (*Pleuracanthus* may also perhaps have belonged to this type.) It is very possible that the Ptyctodonts (Compare HOLMGREN, 1942) and the *Antiarchi* belonged to this type, though without orbital and otic processes. Although the palato-quadrate is very imperfectly known in Arthrodires it may also perhaps have had the same type of relation to the neurocranium. There, however, a suspension by means of a dermal bone (postsuborbital) was added in the Coccosteids. The palatine part also may have had suspensory significance (*Pholidosteus*).

IV. From the imperfectly known Ptyctodont type was probably developed the holostylic condition in the Holocephalians. As there is no otic process at all in Holocephalians (HOLMGREN, 1942) their *holostylic* type must have evolved from a primitive stage at which no otic process occured. The membrane connecting the palato-quadrate with the trabecula and that with the polar cartilage chondrified, the latter forming the basitrabecular process. The upper border of the palato-quadrate fused on to the orbitonasal lamina below and in front of the orbit. Simultaneously the spiracle closed completely and the branchial rays of the palato-quadrate consequently disappeared, only one (or two?) ventral ray (s) being preserved as an otical shelf cartilage comparable with that in sharks (HOLMGREN, 1940, 1942). Postmandibular and ponticular bars are present (HOLMGREN, 1942). The hyoid arch was preserved as a complete arch without suspensorial function but connected by ligaments with the mandibular arch. If the palato-quadrate of a Ptyctodont were to fuse on to the neurocranium, a condition similar to that in the Holocephalians would probably arise (HOLMGREN, 1942).

V a. From an orbitostylic (autodiastylic) condition (III) such as that in lower Acanthodians may have arisen the *hyostylic* in sharks. The hyostyly, already probably prepared in III through a ligamentous connection of the palato-quadrate with the hyoid arch, and the development of a typical spiracular canal, containing a spiracular gill. The palato-quadrate proper has undergone but slight modifications, possibly developing a basitrabecular connection, which, however, never seems to have been articular in sharks, although ontogenetically a well-developed lateral process on the polar cartilage may be present (*Etmopterus,* HOLMGREN, 1940). The ray-bar of the palato-quadrate has probably differentiated into at least three parts. The anterior[1] of these parts forms the not yet suspensorial processus oticus externus (extra-palato-quadrate), which, however, is lacking in *Scyllium* (HOLMGREN, 1940). The second portion of the bar ontogenetically forms a broad process, the processus

[1] It is doubtful whether this part really belongs to the ray-bar.

oticus internus, on the dorso-caudal border of the palato-quadrate, which partly joins on to the neurocranium, there forming the lateral commissure, and partly gives origin to the prespiracular cartilages[1]. The third part develops into a ray (postspiracular cartilage) which mostly fuses on to the lateral surface of the hyomandibula (HOLMGREN, 1940). The articulation between this ray and the palato-quadrate is preserved and forms the palato-quadrato-hyomandibular articulation. As the processus oticus internus is present in modern sharks and rays, as well as in *Dipnoi* and Amphibians, it was certainly also present in some shape in older types of sharks and rays. In Notidanids a very rudimentary membranous lateral commissure is present; *Chlamydoselachus* also has such a commissure, which, however, never chondrifies. Such a commissure must also have been present in the extinct genera, although it could not be preserved in the fossils. Of the lower yaw complex the postsymphysial piece sometimes is preserved as a symphysial cartilage (basimandibular or mandibular copula).

VOL. 24 1943

V b. The hyostyly of the rays (euhyostylic type, GREGORY, 1904) is almost identically developed with that of the sharks, but the orbital process is lacking and the palato-quadrate further removed from the basis cranii. The lower yaw complex is represented by the mandible only, but in *Narke* the posterior "labial" cartilage probably is a part of this complex (HOLMGREN, 1942). With regard to the other characteristics of the skull of rays, I have shown (HOLMGREN, 1940, 1941, 1942) that there are so many very important differences between this group and the sharks that it is not possible to derive them from a common ancestral group. Therefore it seems perhaps necessary to consider that the hyostyly, characteristical of both, is acquired independently in their lines of development.

VI. The *amphistylic* suspension of certain sharks is but a modification of the hyostylic and differs only from the latter through the stronger development of the processus oticus externus (extra-palato-quadrate), which forms an articulation with the postorbital process. Simultaneously with the increase of the external otic process, the internal decreased. (Suspension through a processus oticus externus probably has been acquired independently in Acanthodians and Selachians.)

VII. In rhipidistian Crossopterygians the palato-quadrate articulated with the ethmoidal region and with the polar cartilage region of the neural skull. The former articulation apparently corresponds to the orbital in sharks and *Acipenser* and the ethmoid in bony ganoids and teleosts. The polar cartilage connection undoubtedly is the basitrabecular (basipolar, basipterygial, basal). A lateral commissure (processus oticus internus) was present as in sharks and in most teleostomian fishes. Whether the hyomandibula had anything to

[1] DE BEER (1931) seems to consider that the spiracular cartilage may represent the processus oticus externus, separated from the rest of the palato-quadrate.

do with the suspension of the mandibular arch is not known, but it seems not very likely that so was the case in "*Megalichthys*", as the hyomandibula had a nearly horizontal position (ROMER, 1941) and the posterior, deep part of the palato-quadrate was not very much extended caudally (WATSON, 1925[1]). In *Eusthenopteron*, where the posterior part of the palato-quadrate was drawn out considerably in a caudal direction, a hyomandibular suspension through a symplectic was perhaps present, although not very probable. An epipterygoid bone at the frontal border of the posterior portion of the palato-quadrate probably represents a processus ascendens, which had no articular connection with the neural endocranium. There was no distinct processus oticus externus present.

VIII. In devonian Coelacanthids an ethmoidal and a basitrabecular articulation were present. The mesozoic Coelacanthids were devoid of the latter. In addition the Coelacanthids had a third connection with the neurocranium viz. with the "antotic" process. As this process apparently was situated upon a portion of the skull belonging to the sphenoid part of the skull (basisphenoid) (HOLMGREN and STENSIÖ, 1936), this connection must have been that of an ascending process. This conclusion is sustained by the probable course of the profundus nerve which ran medially to the short posterior part of the palato-quadrate. As the palato-quadrate did not reach the otical region of the skull, no processus oticus externus articulation was developed, but a processus oticus externus nevertheless was present. (STENSIÖ, 1932). A lateral commissure was developed. A long symplectic joining the very long hyomandibula with the articular process of the lower yaw was characteristic to the recent *Latimeria* (SMITH, 1940). The hyomandibula therefore was suspensorial. There was thus two somewhat different types of suspension in the Coelacanthids, the older with basitrabecular articulation, the younger without this joint. In both cases a connection by means of an ascending process with the sphenoid and another (orbital) with the ethmoid region were present.

IX. In primitive ganoid fishes (Palaeoniscids) hyostyly was a common occurrence. But besides the suspension by the hyomandibula there was in lower types also a basitrabecular suspension. A lateral commissure (processus oticus internus) seems always to have been present. A symplectic and a stylohyal were present.

X. The hyostyly of living Ganoids and Teleosts may be a modified shark or paleoniscid hyostyly. The orbital connection (of sharks) has, as already explained (p. 40) shifted frontad to the ethmoidal region, where the palatine part (of sharks) has fused on to the lower side of the ethmoidal plate. In *Lepidosteus* a strong basitrabecular connection is present at a 12 mm stage, but is later replaced by an articular connection between the palato-quadrate and the parotic process of the lateral commissure. This latter connection is

[1] The *Megalichthys* of WATSON is probably not the same as that of ROMER.

developed about at the level of the basal. In front of the basitrabecular connection develops the metapterygoid bone. The posterior upper angle of the palato-quadrate is strongly (without perceptible delineation) connected with the symplectic process of the hyomandibula at the 12 mm stage of *Lepidosteus*. The symplectic subsequently becomes separated from the palato-quadrate through a fissure parallel with the posterior border of the latter, so that the anterior part of the symplectic runs parallel with this border. In prochondrial or chondrified stages the symplectic is never separated from the hyomandibula proper, but it ossifies separately, thereby demonstrating a certain independence of the hyomandibula. In *Acipenser* the cartilaginous symplectic is perfectly separated. When the symplectic process of *Lepidosteus* separates from the palato-quadrate, a posterior upper process of the palato-quadrate arises. This process might possibly represent an external otic process, which has acquired a secondary connection with the parotic process. The internal otic process is represented by the lateral commissure. In *Amia* the upper border of the palato-quadrate has two processes, of which the anterior one is the so-called "pedicle process". It lies anteriorly to the metapterygoid bone and therefore could scarcely correspond to any of the processes in *Lepidosteus*.

XI. The *autostyly* of the Dipnoans must have developed from an early hyostylic condition with orbital (ethmoidal) and basal (basitrabecular) connections and a processus oticus *internus* not yet forming a lateral commissure. In addition there was an ascending process (epipterygoid?) present connected with the sphenoid region of the skull. The idea that the hyomandibula was once suspensorial seems to be a consequence of its strong reduction (*Ceratodus*) or disappearance (*Protopterus, Lepidosiren*), which might have taken place in connection with the palato-quadrate's becoming immovably connected with the neurocranium.

THE HEAD VEINS.

In the morphology of the cranial skeleton the big longitudinal head veins have played an important rôle as many structures have been interpreted according to their location in relation to these veins. It is a great merit of DE BEER to have pointed out the importance of this rôle in a convincing manner and to have worked out a comparative anatomy of the head veins. The primitive head vein is the *vena capitis medialis* [HOCHSTETTER (1888, 1906), RABL (1892)], which in young embryos lies close to the brain and passes medially to the nerve roots and to the auditory vesicle. Frontally it forms the pituitary vein and the orbital sinuses. In sharks the *vena capitis lateralis* subsequently replaces the medial vein in its post-trigeminal course. In a 21 mm embryo of *Heterodontus* the vena capitis lateralis joins the medialis in front of the facial nerve and is connected with it anteriorly and posteriorly to the

VOL. 24 1943

glossopharyngeal nerve and posteriorly to the first branch of the vagus nerve. In a *Scyllium* embryo, reconstructed by DE BEER, 4 vagus branches are seen enclosed in loops by the lateralis vein, but there is no loop around the glossopharyngeal nerve. In a 33 mm *Scyllium* the vena capitis lateralis runs dorsally to the facialis, glossopharyngeus and vagus nerves and dorsally to the hyomandibula but inside (ventrally to) the levator muscle of the hyomandibula, and this may be the general arrangement in sharks and rays (DE BEER, reconstruction of *Torpedo marmorata,* 1926).

In *Acipenser* (DE BEER, 1925) the jugular vein or vena capitis lateralis at a 5-day stage of *Acipenser stellatus* is said to run "median to the branches of the trigeminal nerve, lateral to the palatine and the hyomandibular branches of the facial, lateral to the glossopharyngeal and median to the vagus". The vena capitis lateralis in *Acipenser* thus does not run quite the same way as in sharks and rays.

In addition to the reducing vena capitis medialis there are in *Amia* two big longitudinal veins described by DE BEER: the vena capitis lateralis and the "secondary vein". The secondary vein arises earlier than the vena capitis lateralis. In a 6 mm embryo it is found running dorsally to the facial nerve but ventrally to the glossopharyngeal and vagus nerves. In 8 and 11 mm embryos, however, it proceeds dorsally to the facialis and glossopharyngeus but ventrally to the vagus. Frontally, the secondary vein runs into the remaining part of the vena capitis medialis, thus carrying the pituitary vein and the orbital sinuses. The secondary vein is said to have a branch entering the canal of the hyomandibular nerve through the hyomandibula. The vena capitis lateralis of the 11 mm stage runs dorsally to the facialis, glossopharyngeus and vagus roots. The lateralis and the secondary vein run medially to the hyomandibula, the lateralis outside, the secondary vein inside the "hyomandibular muscle". At the 50 mm stage reconstruction the vena capitis lateralis is seen to diverge considerably from the secondary vein in front of the hyomandibula.

In *Salmo* the secondary and lateralis veins run as in *Amia,* except that the secondary vein goes ventrally to the hyomandibularis and also ventrally to the glossopharyngeal nerve instead of dorsally to them. The vena capitis lateralis in a 20 mm embryo runs caudally into the lateral cutaneous vein.

Finally, in *Cottus bubalis,* according to DE BEER, there is an extra vein present laterally to the hyomandibula and "running into the sinuses in front". Posteriorly it "joins the other two veins in the region of the glossopharyngeal".

In a 13 mm *Ceratodus* the head vein "is therefore lateralis as far forward as the facial, then medialis", but in the figures it is behind the facial, alternately labelled vena capitis medialis and secondary vein. There thus seems to be some doubt as to its homology.

In *Polypterus,* according to MOY-THOMAS' (1933) reconstruction, the vena capitis lateralis is present, connected (by means of a medialis posterior) frontally with the dorsal and ventral orbital sinuses.

It is fairly clear from this summary that the homologies could not be established with any certainty merely from the relations of the veins to the nerve roots, shifting being rather common, and that fresh investigations are necessary for determining the homologies, if they can be determined at all.

The head veins in Squalus acanthias embryos.

In a series of descriptions and diagrammatic reconstructions DE BEER (1924) tried to demonstrate the early development of the head veins in sharks. At early stages the only cranial vein (in *Scyllium*) is the vena capitis medialis. It runs ventrally to the big cranial nerve roots. Behind the oculomotor nerve the veins of the two sides communicate by means of the pituitary vein. Between the glossopharyngeal and vagus ganglia a part of the "vena capitis lateralis" appears as a short rudiment. Later it seems to consist of an anterior portion lying laterally to the auditory sac and dorsally to the hyomandibular nerve, and a posterior connected behind the vagus nerve with the vena capitis medialis. This latter portion lies ventrally to the vagus nerve and is called the "secondary vein". It is said later to acquire its "definitive external position" in relation to the vagus nerve root. As the next stage of development DE BEER has interpolated a 21 mm stage of *Heterodontus,* where the vena capitis lateralis (the transformed "secondary vein") is said to be developed externally to the facialis, glossopharyngeus and the first portion of the vagus nerves. It is connected with the vena capitis medialis anteriorly to the facialis, anteriorly and posteriorly to the glossopharyngeus and posteriorly to the first portion of the vagus nerves. In front the capitis medialis forms the orbital sinuses. The medialis and the orbital sinuses lie internally to the ventral cranial nerves (oculomotor, trochlearis). At a later stage the medialis becomes reduced from behind. "The lateralis is now the functional vein of the head." It loops around nearly all vagus branches. "Anteriorly the superior orbital sinus connects with the medialis behind the maxillo-mandibulary branch of the trigeminal, forming a lateralis in this region. The hypophysial vein branches off from the medialis medially to the trigeminal." At the 26 mm stage it is complete, lying externally to all the big cranial nerves. The trigeminal and the facial nerves are separated by the only remaining part of the medialis, from which issues the hypophysial vein. At a 33 mm stage, when cartilage is developed, the lateralis runs outside the hyomandibula but inside the hyomandibular muscle. The hypophysial vein lies in front of the pila antotica. From the accompanying very diagrammatic reconstruction in his

VOL. 24 1943

figure 9 the maxillo-mandibular nerve would appear to separate the upper and lower orbital sinuses.

The development of the cephalic circulatory system of the early embryos of *Squalus acanthias* has been studied by SCAMMON (1911). The chief result of his investigation is that according to him the vena capitis medialis is differentiated from the anterior end of the aortic loop i.e. that part where the dorsal aorta (carotis interna) is joined by the mandibular artery (pseudobranchial artery). At a 4.8 mm stage the vena capitis medialis forms a short twig at the frontal border of the loop. At the 5.2 mm stage this twig has a small ventral branch. At the 7.5 mm stage the vena capitis medialis forms a short protruding anterior continuation of the cephalic sinus (anterior portion of the dorsal aorta). At this stage the vein has three short ventral branches. At the 11.5 mm stage the vena capitis medialis and the aortic system have separated. An arteria ophthalmica magna runs into the mandibular artery near its frontal end. As I cannot support any of the results of SCAMMON's investigations, I will now describe the development of the cephalic circulatory systems as I have found it. My decription is based upon graphical reconstructions from serial sections of the following stages: 5.7, 7.5, 9.5, 12.5, 18 and 38 mm.

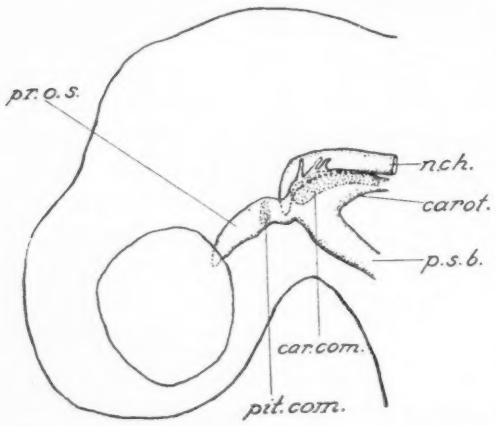


Fig. 53. *Squalus acanthias.* Embryos 5.7 mm. Reconstruction of the bloodvessels of the head.

At the 5.7 mm stage (fig. 53) the cephalic vascular system consists of the mandibular artery joining on to the dorsal aorta and a frontal process, the primary orbital sinus, issuing from each aortae. The frontal ends of the aortae are connected with one another by means of a broad commissure (the commissure of the carotis interna). From the dorso-frontal border of the aorta three small branches extend dorsad and dorso-caudad. The frontal processes of the aortae extends towards the eye. Near its base the processes are united medially by a broad commissure (the futur pituitary vein). The processes I will call the *primary orbital sinuses.*

At the 7.5 mm stage (fig. 54) the primary orbital sinus has prolonged considerably in front, there sending off two short and slender branches of which the one is directed dorsally to the eye tube, the other ventrally to it. These branches are the rudiments of the supraorbital and infraorbital veins or sinuses respectively. The three short dorsal twigs are developed as at the

preceding stage. From the basal half of the primary orbital sinus a short posterior branch is given off, which runs mediad below the anterior border of the premandibular commissure. The pituitary and the carotic commissures are as at the preceding stage. The anterior end of the primary orbital sinus is connected with the mandibular artery through a frontally wide, caudally very rudimentary bloodvessel, the rudiment of the *arteria ophthalmica magna*. At the ventro-lateral surface of the brain, dorsally to the anterior aortic portion, two short rudiments of bloodvessels are found. These lie in line with one another forming the rudiments of a longitudinal vessel. This vessel is the future vena capitis medialis.

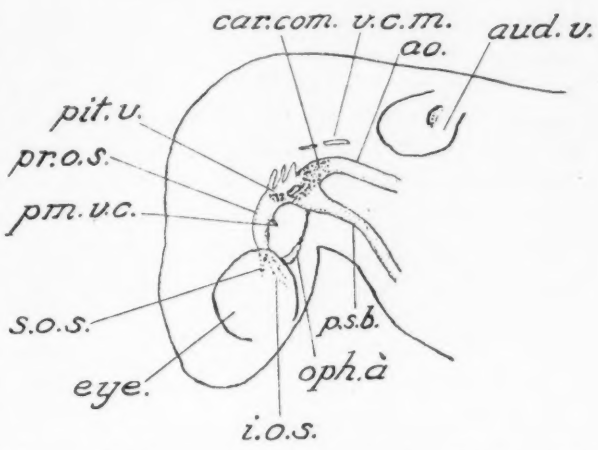


Fig. 54. *Squalus acanthias*. Embryo 7.5 mm. Reconstruction of the bloodvessels of the head.

At a 9.5 mm stage (fig. 55) the orbital rudiments are principally as at the preceding stage, but the rudiments of the vena capitis medialis have joined with one another forming a continuous vein; frontally it has connected up with the two most caudal of the dorsal twigs of the primary orbital sinus. The anterior end of the medialis forms in front of the twigs the rudiment of the intermedian cerebral vein. On the orbital sinus two anterior (dorsal) branches from the brain have arisen. The posterior (ventral) branch of the orbital sinus, which in the preceding stage was found below the anterior border of the commissure of the premandibular somite, now has met its vis à

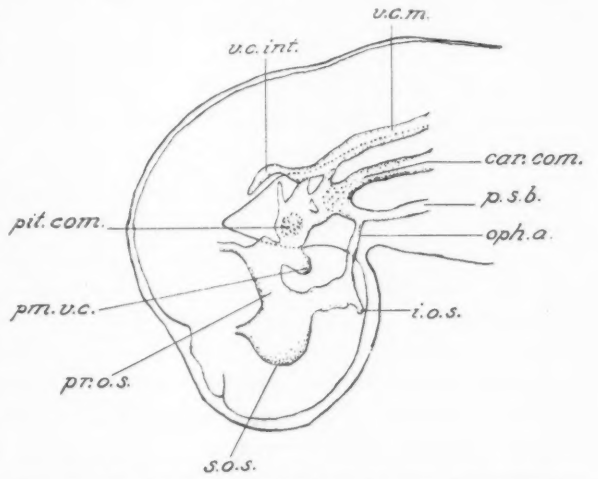


Fig. 55. *Squalus acanthias*. Embryo 9.5 mm. Reconstruction of the bloodvessels of the head.

vis forming a commissure. The pituitary commissure is as in the preceding stage.

It is clear from the description of the 7.5—9.5 mm stages given in the preceding lines 1. *that the hypophysial and orbital veins form a venous unit,* and 2. *that the vena capitis medialis and the vena cerebralis intermedia are genetically another unit.* Thus the interpretation of the orbital veins as part of the vena capitis medialis is decidedly incorrect.

But these early stages of development are of great interest from another point of view, as they show that the orbital vein system originally forms a part of the arterial system, constituting an arterial arch in front of the mandibular, thus a premandibular arch. Of this arch the ventral portion later will develop into the arteria ophthalmica magna, whereas the rest of this arch (the efferent premandibular artery) develops into the orbital venous sinus, so that this artery thus represents the afferent premandibular artery. In his very valuable paper on the pseudobranchial and carotid arteries of bony fishes and *Amia* ALLIS (1912) has with admirable intuition expressed the opinion that the ophthalmica magna is a persisting premandibular artery. But as in the adult this artery originates from the lateral dorsal aorta, he concluded that the ophthalmica magna represents the *efferent* artery of the prebranchial arch. According to ALLIS the choroid gland should be the glomus of the premandibular arch.

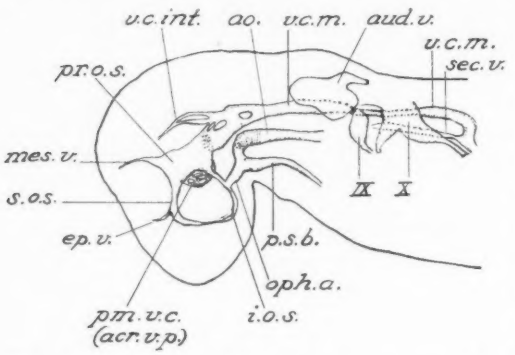


Fig. 56. *Squalus acanthias.* Embryo 12.5 mm. Reconstruction of the bloodvessels of the head.

At the 12.5 mm stage (fig. 56) the arterial and venous systems have separated from one another dorso-frontally, but the connection by means of the ophthalmic artery is still present. The rudiments of the dorsal and ventral orbital veins have grown out frontad and meet one another in front of the eye, so that a circumocular vein ring has arisen. The vena capitis medialis is joined on to the primary orbital sinus and the intermedial cerebral vein seems to issue from this sinus. Caudally behind the N. vagus the vena capitis medialis joins the cuvierian duct. From the upper end of this duct has arisen a longitudinal vein directed frontally. This vessel runs medially to the vagus ganglion and reaches to a level somewhat posteriorly to the glossopharyngeal ganglion. This new vein corresponds to the "secondary vein" described by DE BEER. No traces of the vena capitis lateralis are present as yet.

In the reconstruction of stage J of *Scyllium* published by DE BEER a small

rudiment of the secondary vein is present, but there is also a rudiment of the vena capitis lateralis between the glossopharyngeal and vagus nerves. In his stage K, where the secondary vein has grown longer, the rudiment of the vena capitis lateralis was found between the facialis and the glossopharyngeus outside the auditory vesicle. These two stages might possibly indicate that the vena capitis lateralis has arisen in situ by means of independent rudiments. In my material of *Scyllium* I have not found any corresponding stages of development, probably because I had not a sufficient number of stages at my disposal. At a 12 mm stage of *Scyllium*, however, where no lateralis is indicated, the secondary vein extends to a point in front of the glossopharyngeal nerve and carries short dorsal branches behind the glossopharyngeus and the vagus nerves, which may perhaps indicate that the development of the lateralis, occasionally at least, follows the method that in the following is found to occur in *Squalus*.

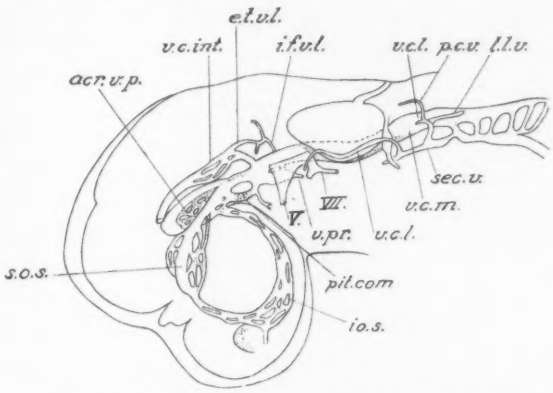


Fig. 57. *Squalus acanthias*. Embryo 18 mm. Reconstruction of the vein system of the head. Plexa diagrammatically represented.

For the understanding of the development of the vena capitis lateralis the 18 mm stage of *Squalus* is of great interest (fig. 57). At this stage the medialis is still present as the principal head vein; its frontal portion, however, has developed into the intermedial cerebral vein. The aortic portion of the vein system has separated completely from the artery system and has developed vein plexa in the acrochordal mesenchyme of the brain flexure and around the eye (superior and inferior orbital sinuses). A caudally directed venous process (v.pr.) inside the maxillo-mandibular portion of the facialis nerve is about to join the most anterior portion of the vena capitis lateralis coming down from the dorsal side of the hyomandibular nerve. The vena capitis lateralis is present from a point in front of the hyomandibular nerve to behind the glossopharyngeal ganglion, where it bends down to join the "secondary vein". Behind the vagus complex the secondary vein has a dorsal branch which bends round behind this nerve, extending its tip frontad on a level with the vena capitis lateralis. At a stage[1] in which the development of the veins is somewhat more advanced this tip joins the next caudal portion of the vena capitis lateralis. From the convexity of the postvagal branch of

[1] There is a wide variability in the development of the veins, and the bodylength cannot be used as an indicator of the degree of development of the veins.

VOL. 24 1943

the secondary vein issues the rudiment of the lateral line vessel. From the horizontal part of the hooklike postvagal branch of the secondary vein a thin branch runs dorsad and then frontad along the outside of the vagus ganglion (p.e.v.) and the brain. This branch is the posterior cerebral vein. At the actual 18 mm stage (fig. 57) the "secondary vein" has a commissure with the vena capitis medialis below the vagus nerve, and then follow 5 segmentally arranged connections between these veins, of which the two caudal join together before joining the secondary vein. From this stage it seems to be fairly clear that the vena capitis lateralis arises, partly at least, from branches of the secondary vein bending round the ganglia and forming loops around them. At a 20 mm stage the vena capitis lateralis has become connected with the vena capitis medialis by commissures before and behind the glossopharyngeus, the latter being very weak. In respect of these commissures the 20 mm stage corresponds to the 21 mm stage of *Heterodontus* as illustrated by DE BEER. But in addition, the secondary vein is present inside the glossopharyngeus and vagus and its connections with the lateralis are still present. These nerves are thus enclosed partly between the lateralis and the medialis, partly between the lateralis and the secondary vein. The chief difference between this 21 mm *Heterodontus* and the 20 mm (and 22 mm, 23 mm) stage of *Squalus* seems to be that in the latter the secondary vein is always present, whereas it is lacking in *Heterodontus*. DE BEER believes that in this *Heterodontus* the secondary vein has become the lateralis, an interpretation that does not seem to be in accord with the *Squalus* conditions.

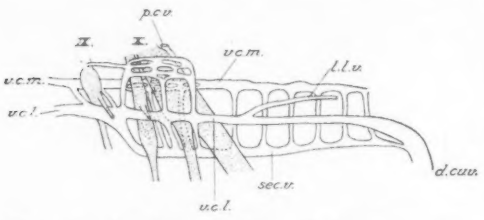


Fig. 58. *Squalus acanthias*. Embryo 22 mm. The principal headveins, diagrammatically reconstructed.

In a 22 mm *Squalus* embryo (fig. 58) the vena capitis lateralis is completed and forms a continuous vessel from the cuvierian duct caudally to the orbital sinuses in front. At this stage the secondary vein is also present ventrally to the vagus nerve, caudally joining the ductus cuvieri, Frontally it joins the lateralis behind the glossopharyngeus nerve. Moreover, similar connections are present behind the first and second portions of the vagus nerve. In front of and behind the glossopharyngeus nerve the lateralis is connected with the medialis. Outside the vagus ganglion a venous plexus is developed, which is connected with the lateralis at 3 points, of which the hindmost lies behind the second portion of the vagus. This plexus is continuous with a similar plexus on the inside of the vagus ganglion, and this latter part is connected with the vena capitis medialis. The vagus ganglion is thus overspun with veins. A vein (posterior cerebral vein) comes to this plexus from the brain.

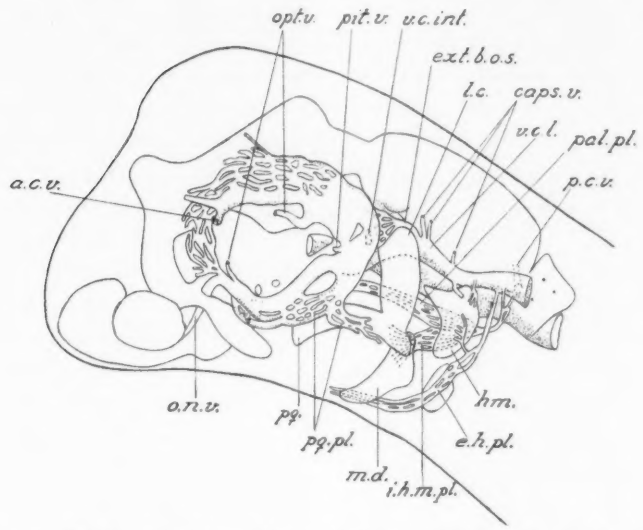


Fig. 59. *Squalus acanthias*. Embryo 38 mm. Venous system of the head.

Medially to the vagus ganglion two vertical vein branches connect the secondary vein with the medialis, and behind the vagus there are 6 similar branches (intersegmental veins) in front of the ductus cuvieri. No appreciable changes have taken place in the anterior parts of the venous system of the head. The vena capitis lateralis has now met the process (described in the 18 mm stage) from the aortic part below the maxillo-mandibular nerve complex and in front of the hyomandibular nerve. Where the lateralis bends down below the trigeminal, it sends off some small branches which frontad run dorsally to the maxillo-mandibular nerve. These branches later join the posterior superficial portion of the orbital sinus from the inside. In the 26 mm embryo of *Scyllium* this communication is represented by a fairly wide vessel which joins the upper orbital vein in front of the trigeminal. I also found this communication in my material of *Scyllium*. As DE BEER says that the lateralis lies externally to all the nerves except the ophthalmic branches of the trigeminal and facial, it is obvious that he has mistaken this communication for part of the lateralis proper. Its development in *Squalus* demonstrates its secondary character. In the 33 mm stage of *Scyllium* described by DE BEER the lateralis is moreover illustrated as running ventrally to the maxillo-mandibular nerve. My 32 mm specimen is very different and agrees in principle with the 38 mm stage of *Squalus*.

At the 38 mm stage of *Squalus* (fig. 59) the vena capitis lateralis is the principal head vein. The secondary vein and the medialis are not discernible any longer as longitudinal head veins, the medialis being represented by the

VOL. 24 1943

intermedial cerebral veins, and the secondary vein at least by venous loops around the anterior roots of the vagus. Venous plexa have developed outside the hyomandibula and ceratohyal extending frontad to the inside of the lower jaw. These plexa open into the vena capitis lateralis in front of the posterior cerebral vein. At almost the same point a big vein plexus running frontad in the soft palate mesenchyme begins with a number of branches. From this plexus a part is detached to be spread on the inside of the hyomandibula. The dorsal part of the outside of the hyomandibula is partly covered with rami belonging to short vein branches opening in the lateralis dorsally to the hyomandibula. From the dorsal border of the lateralis branches are sent off to the outside of the auditory capsule. Where the lateralis is about to enter inside the lateral commissure it sends off a dorsal branch, which forms a plexus on the outside of this commissure and on the dorsal side of the maxillo-mandibular nerve. Ultimately this plexus, in part at least, joins the orbital sinus.

In front the lateralis receives the intermedial cerebral vein and then opens into the orbital sinus, which overlaps the frontal part of the lateralis on its outside. Caudally the orbital sinus forms a high sac on the inside of the caudal part of the eye. It is pierced by the rectus externus muscle of the eye. The upper and the lower orbital veins run out from this sac-like portion. The upper orbital vein on its dorsal border is closely connected with a plexus of small vein branches, which continues in front of the eye to connect with the anterior part of the lower orbital vein. The lower orbital vein is also connected with a vein plexus. This plexus lies partly on the outside of the palato-quadrate, partly ventrally to the orbital vein proper. The plexus part on the outside of the palato-quadrate is connected by means of a wide sinus with the saclike part of the orbital sinus. The hypophysial vein runs out from the point where the dorsal and ventral orbital veins meet. An anterior cerebral vein is present and also an upper and a lower branch for the eye.

In the 48 mm stage the vein system is far more complicated owing to the rich development of venous plexa. Of these I will mention here only a plexus on the inside of the palato-quadrate and the mandibula. Caudally this plexus is connected with the medial plexus inside the hyomandibula, and thus with that of the palate as well. At the mandibular articulation part of this plexus also comes outside the palato-quadrate. The plexus of the palate, moreover, has been connected with the lower orbital sinus somewhat in front of the pituitary vein. This connection forms frontally a venous commissure bordering the palatine plexus in front. This commissure corresponds to the strong commissure found in rays (*Raja, Torpedo, Urolophus*) (Fig. 18 HOLMGREN, 1942).

The head veins in Acipenser embryos.

In his paper on the development of the skull in sturgeons DE BEER (1925) has also paid some attention to the head vein. At a five-day stage of *Acipenser stellatus*, where the chondrocranium is fairly well developed, the jugular vein runs "median to the branches of the trigeminal nerve (and to the buccal and otic branches of the facial), laterally to the palatine and the hyomandibular branches of the facial, laterally to the glossopharyngeal and median to the vagus". In the figures of sections accompanying the text this vein, level with the vagus ganglion, is labelled vena capitis medialis. According to the same figures a vena capitis lateralis exists at the level of the glosopharyngeal ganglion and the facial nerve. Further frontally where it is found inside the ramus mandibularis V but outside the palatine VII nerve the jugular vein is labelled vena capitis medialis. At the 10½ day stage the head vein follows about the same course as that in the five-day stage. It has a big branch behind the glossopharyngeal root and the suprapharyngobranchial of the 1st branchial arch. This vein branch bends frontad below (inside) the glossopharyngeal ganglion, after which it was not followed any more. SEWERTZOFF's (1928) description of the development of the skull of *Acipenser ruthenus* does not contain any particulars about the venous system. As it seems to be very unlikely that a vena capitis medialis should form the backward continuation of the lateralis it is quite necessary to reinvestigate this system from embryological points of view before using it for comparative anatomical purposes.

In my collection the youngest stage available for reconstruction is a 9 mm stage of *Acipenser Gyldenstaedti*, where there is still no trace of cartilage present, and the parachordals are only foreshadowed by a mesenchyme about to become transformed into procartilage. The mandibular arch is represented by a similar but denser mesenchyme, as is also the case with the mesenchymatic parts that are later to be used in the formation of the barbels. This stage, though very much less developed than the 4-day stage of DE BEER is, however, too old for demonstrating the earliest part of the development of the veins; nevertheless it is of great value for our understanding of the later stages.

Acipenser Gyldenstaedti, 9 mm. This stage is too old to allow any explanation of the origin of the anterior portion of the venous system, whether it originates from the aortic system, as in sharks, or not. The principal veins behind the trigeminal ganglion are the *vena capitis medialis* and the secondary vein (fig. 60). The vena capitis medialis joins the cuvierian duct behind the vagus. Frontad it extends inside the auditory vesicle and inside all nerves, joining up with the secondary vein just in front of the facialis ganglion. The secondary vein, posteriorly connected with the medialis and with the ductus

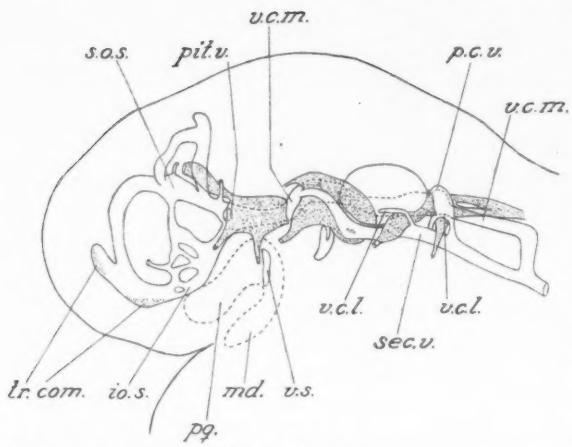


Fig. 60. *Acipenser Gyldenstaedti*. Embryo 9 mm. Venous system of the head. Plexa diagrammatically represented.

cuvieri, runs frontad inside (below) the vagus and the glossopharyngeal ganglia but outside (above) the facialis ganglion. At the present stage this vein has three branches, one in front of and one behind the first portion of the vagus ganglion and one behind the glossopharyngeal ganglion. The two first mentioned branches join dorsally to the vagus. From the join a broad sinus extends dorsad, reaching dorsally to the vagus and the glossopharyngeal nerve roots and subsequently communicating with the medialis in front of these nerves. This sinus represents the rudiment of the posterior cerebral vein and the two branches connecting it with the secondary vein form a loop around the lateral (lower) part of the vagus ganglion. The post-glossopharyngeal branch bends over the glossopharyngeal ganglion in a frontal direction.

From the point at which the secondary vein joins the medialis, the head vein runs a long distance inside the trigeminal ganglion to the orbit before branching. At the posterior margin of the eye lies the hypophysial vein branch, and there a dorsal and a ventral orbital vein (or sinus) branch off. Details of these sinuses will not be described, but the reader is referred to the figure, in which, however, the lower plexus is quite diagrammatical. The two orbital sinuses meet in front of the eye, thus forming a ring round the eye. This ring is connected with that of the other side by two commissures, one behind the nasal sac and one in front of the medium part of that sac. In an *Acipenser Gyldenstaedti* of 8 mm body-length the postfacial portion of the head veins is developed in the following way. The vena capitis medialis is a very wide vessel. The posterior part of the secondary vein is branchless and wide as far as to the anterior part of the glossopharyngeus, but here it narrows very much, ultimately becoming as fine as thread, in the present series of sections on short stretches not with any certainty discernible.

The 9 mm stage of *Acipenser Gyldenstaedti* is of great interest from different points of view. 1) It represents probably the only case in recent fish where a secondary vein is present in its entire length, 2) it enables a comparison to be made with the conditions in young shark-embryos and 3) it seems to be essential for our understanding of older stages of *Acipenser*.

1) This thesis must be reserved for a discussion on the conditions in embryos of other ganoids and in teleost embryos (*Salmo*).

2) The 8 mm stage of *Acipenser* differs from the 12.5 mm stage of *Squalus*, as in the latter the secondary vein already ends at the level of the posterior part of the glossopharyngeal ganglion, whereas in *Acipenser* it continues from that point to join the medialis in front of the facial ganglion. In the part lying in front of the glossopharyngeus the secondary vein of a 8 mm *Acipenser* is threadlike and seems in part to be not fully formed.

The 9 mm stage of *Acipenser* shows striking resemblances with the 18 and 22 mm stages of *Squalus* in the glossopharyngeus and vagus region. In the *Squalus* of 18 mm a branch from the secondary vein rises behind the 1st vagus ganglion. This branch bends frontad dorsally to the ganglion and later forms a loop around the lower part of it. From a corresponding branch in the 9 mm *Acipenser* a similar loop is developed. In the *Squalus* a dorsal branch from the dorsal portion of the loop joins a longitudinal vessel running dorsally to the root of the vagus and glossopharyngeus, joining the medialis in front of the ganglion. In *Acipenser* a vein sinus issues from the loop joining the medialis in a similar way. In *Acipenser* a branch issues from the secondary vein behind the glossopharyngeal ganglion and bends over its dorsal side. In the 18 mm *Squalus* a similar branch is also present but it runs frontally into the rudiment of the vena capitis lateralis outside the auditory sac. According to DE BEER this portion of the lateralis of *Scyllium* is said to originate separately. Perhaps this may also be the case in *Squalus*. In *Squalus* the dorsal parts of the loops join with one another forming the lateralis. It seems plausible therefore that the corresponding parts in *Acipenser* may represent the vena capitis lateralis.

Accordingly at the 9 mm stage of *Acipenser Gyldenstaedti* the following head veins are apparently present: 1. The vena capitis medialis, 2. a complete secondary vein and 3. two sections (glossopharyngeal and vagal) of the vena capitis lateralis. The vena capitis medialis and the secondary vein join in front of the facialis ganglion.

At the 9 mm stage there is a complete venous ring round the eye, the dorsal and the ventral orbital sinuses being connected in front of the eye. The proximal portion of the lower orbital sinus forms a plexus below and inside the eye and inside the mesenchyme of the future barbels. Outside the posterior part of the palato-quadrate is an isolated venous sinus.

Unfortunately my next stage of *Acipenser* is a little too far advanced to allow of a complete comparison with the 9 mm stage; moreover, it belongs to *Acipenser stellatus*. Nevertheless it is possible to make some important observations using a 10 and a 13 mm *stellatus* and comparing them simultaneously with a 14 mm *Gyldenstaedti*.

VOL. 24 1943

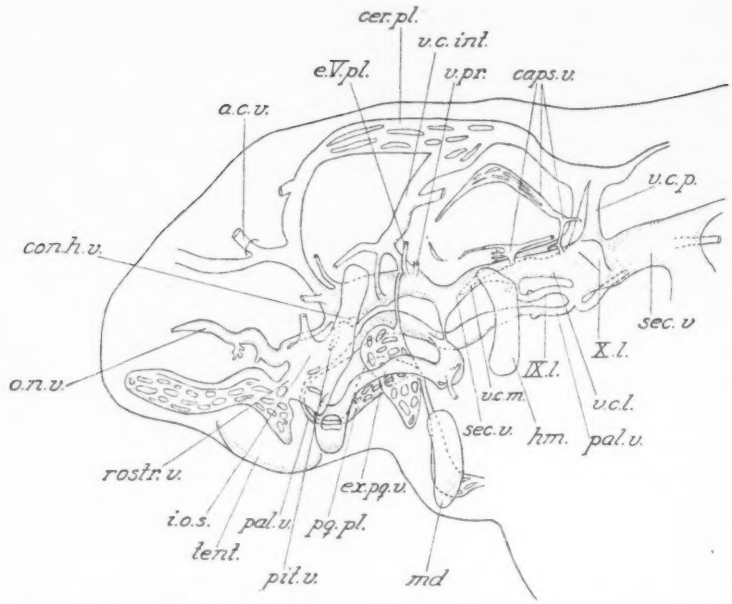


Fig. 61. *Acipenser stellatus.* Embryo 13 mm. Venous system of the head. Plexa diagrammatically represented.

However, at the 10 mm stage of *stellatus,* which is a little more advanced than the 9 mm stage of *Gyldenstaedti,* the loops over the dorsal side of the vagus and glossopharyngeus ganglia are complete and the looping-over part nearly as wide as the secondary vein proper. In addition, the two loops are thrown together so that there are only three short perpendicular branches bridged over by the lateralis. The lateralis thus extends from behind the vagus to a point in front of the glossopharyngeus.

At the 13 mm stage of *stellatus* development has proceeded much further (fig. 61). It is no longer possible to follow the vena capitis medialis as a longitudinal tube, as it has become desintegrated to form part of a venous plexus covering the sides and the basis of the brain. Only from the level of the hyomandibula to the level of the vagus nerve is a vein present, lying exactly in the position of the medialis of young stages. This vein does not, however, join the secondary vein, but turns upwards at the level of the vagus nerve to join a vein sinus running dorsally to the nerve roots close to the brain. This vessel forms a dorsal border part of a venous plexus covering the sides of the brain and extending frontad to the orbital region. The posterior, the intermedium and two anterior cerebral veins connect the cerebral plexus to the head vein system.

The head vein runs ventrally to the vagus, then dorsally to the glossopharyngeus and to the facialis, then turning ventrally (medial) to the

trigeminal complex. This vein has thus shifted in the glossopharyngeal region from a position ventrally to the nerve to a position dorsally to it; elsewhere it runs as the secondary vein of the 9 mm stage. At the 10 mm stage we observed that the dorsal part of the glossopharyngeal loop had widened almost to the same width as the secondary vein of the corresponding section. At the 13 mm stage the dorsal part of the loop has become very wide, whereas the corresponding portion of the secondary vein has narrowed. The dorsal loop portion has now become part of the head vein. In consequence the head vein, counting from behind, consists 1) of a vagal portion belonging to the secondary vein, 2) of a glossopharyngeal portion consisting of a vena capitis lateralis, and 3) of an anterior portion, which again is the secondary vein.

The vagus loop is always present, but instead of being located on the dorsal side of the secondary vein it has shifted laterad. This shifting has resulted in the posterior cerebral vein (the dorsal vein sinus of the loop) now issuing from the secondary vein proper.

From the posterior part of the glossopharyngeal loop, i.e. from the secondary vein, a big vein branch, here called the "palatine vein", runs out in a frontomedial direction. This vein soon approaches the ventral midline dorsally to the mouth cavity and runs frontad parallel with its vis à vis, with which it is connected by a weak commissure behind the hyomandibula. The palatine vein is very wide as far as to the orbit, where it narrows extremely, bending down to the palato-quadrate symphysis, where it turns backwards joining a great venous plexus inside the palato-quadrate. This plexus is connected by means of a vein branch with a plexus lying inside the mandibula.

On the outside of the posterior part of the palato-quadrate lies a large venous plexus. This plexus is connected with a big vein, the extrapalato-quadrate vein, lying on the outside of it, and extending along the entire palato-quadrate outside the plexus. Caudally this vein turns dorsad and frontad to join the palatine vein. Frontally the extrapalato-quadrate vein has a connection with the lower part of the orbital sinus and caudally also with the secondary vein in front of the hyomandibula. The extrapalato-quadrate plexus also has a connection with the secondary vein at the base of the intermedial cerebral vein. From the upward bend of the extrapalato-quadrate vein, two short branches proceed caudad. They soon terminate, however. From the head vein issue a number of branches. On a level with the frontal portion of the vagus complex the short and stout cerebral vein arises. In front of it a rather slender branch goes in a dorsolateral direction. It has three branches, one short basal frontal and two apical, a caudal and a frontal, of which the caudal soon terminates inside the skin, whereas the frontal one describes a curve on the outside of the auditory capsule, terminating at its frontal part directed towards the upper part of orbital sinus. Just behind the hyomandibula

VOL. 24 1943

the head vein (lateralis) hos another dorsal branch, which sends out a number of very fine branches frontad, and a wider branch caudad, all lying on the ventrolateral border of the auditory capsule. In front of the hyomandibula a medial branch runs out and ends on the inside of the hyomandibular ganglion. Immediately behind the intermedial cerebral vein a rather wide branch enters the protractor muscle of the hyomandibula, and in front of it a plexus (represented in the figure by a vein branch only) between the head vein and the intermedial cerebral vein covers the outside of the trigeminal II ganglion.

The orbital portion of the head-vein system has somewhat altered, and could not be compared in any detail with that of the 9 mm stage. This is due partly to the development during the 13 mm stage of a big orbital sinus. There is no doubt about its originating in connection with the lower orbital vein. At the 9 mm stage the lower orbital vein is more or less plexiform, at the 13 mm stage it has developed into a broad sinus behind the eye, extending dorsally up to the level of the upper margin of the eye. Frontally the sinus extends into two wide veins: the dorsal orbitonasal vein and the ventral rostral vein, which forms an abundant plexus below the future rostrum. The orbital sinus is connected with the head vein behind the hypophysial vein. The upper orbital vein does not form a sinus. For details see the figure, in which, however, the plexa are represented diagrammatically.

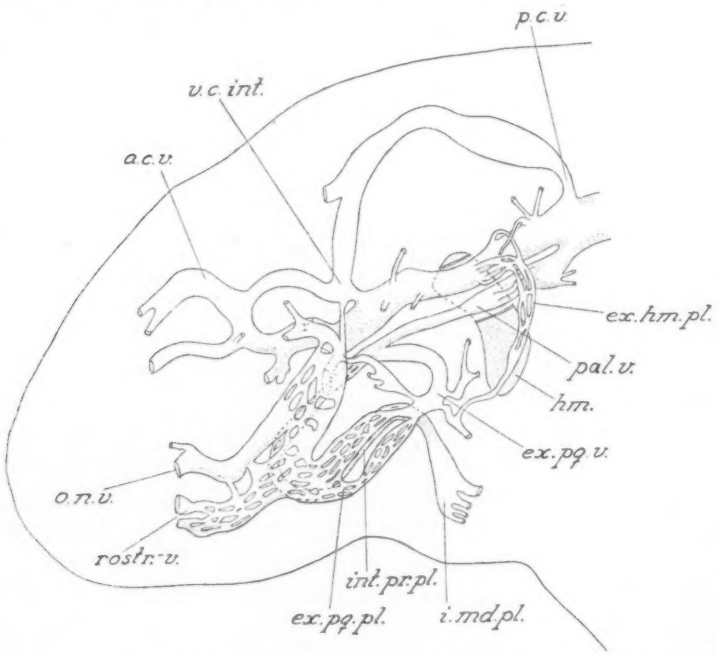


Fig. 62. *Acipenser Gyldenstaedti.* Embryo 14 mm. Venous system of the head. Plexa diagrammatically represented.

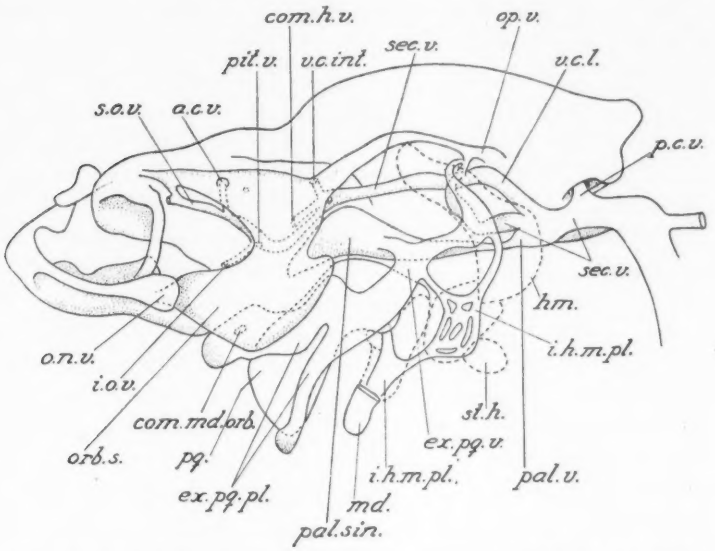


Fig. 63. *Acipenser ruthenus*. Embryo 14 mm. Venous system of the head. Plexa diagrammatically represented.

In a 14 mm embryo of *A. Gyldenstaedti* (fig. 62) the development had advanced slightly further than in the 13 mm *stellatus*. As, however, these embryos belong to different species it is not possible always to tell what is due to specific differentiation and what to advancing development. I would only point out therefore that the venous plexus on the lateral side of the hyomandibula must be considered to be the result of a development of the vein branches issuing from the head vein just behind the hyomandibula. A connection between the intermedium cerebral vein and the orbital sinus in *Gyldenstaedti* may perhaps represent a specific character, partly corresponding to the connection between the same vein and the extramandibular plexus in *stellatus*.

In a 14 mm embryo of *A. ruthenus* (fig. 63) the development is far more advanced than at the preceding stages, *ruthenus* being a small sturgeon species. The 14 mm *ruthenus* is somewhat less developed than the 10½ day embryo of *stellatus* reconstructed by DE BEER, and also rather less developed than the *ruthenus* (without measurements) of SEWERTZOFF (1928). The 14 mm stage of *ruthenus* is characterized by the great development of the peripheral plexa and sinuses inside the hyoid arch and the mandibula, and outside the palato-quadrate. The orbital sinus is also considerably more developed than in the preceding stage. The dorsal branch of the head vein which at the preceding stages lies somewhat in front of the posterior cerebral vein now lies a little behind the hyomandibula. It has three branches, of which an

VOL. 24 1943

upper posterior forms a plexus in the opercular fold and outside the hyomandibula. An upper anterior branch runs frontad on the outside of the auditory capsule and opens frontally into the dorsal part of the orbital sinus. The third branch turns inside the hyomandibula. It partly forms a great plexus inside the symplectic, partly it is connected with the upper part of the extrapalato-quadrate vein. The plexus communicates partly with the extrapalato-quadrate vein, partly with an internal mandibular sinus, which is connected with the external palato-quadrate sinus. The orbital sinus consists of a dorsal venous sac extending to the frontal end of the orbit and a big ventral sac narrowing frontally to form the orbitonasal vein. The dorsal orbital vein, independent of the dorsal sinus, runs through the dorsal part of the orbit and branches off the hypophysial and the anterior cerebral veins. The head vein has a rather narrow communication with the orbital sinuses. An intermedium cerebral vein is also present. There is a communication between the extrapalato-quadrate sinus and the lower part of the orbital sinus. In the actual specimen no inferior orbital vein is present[1], but in slightly younger and older embryos such a vein was found branching off from the superior orbital vein just in front of the hypophysial vein. Frontally, the palatine veins widen considerably to form big sinuses, which are joined by a broad commissure. The glossopharyngeal loop is formed, as in the 13 mm *stellatus*, by the secondary vein.

The head veins in Amia embryos.

The development of the head veins in *Amia* has been described by DE BEER. According to him the vena capitis medialis and the secondary vein are present in a 6 mm embryo. The latter leaves the medialis in front of the facial ganglion, and then runs laterally (dorsally) to the facialis nerve but medially to the glossopharyngeus as well as to the vagus nerves. At the 7 mm stage "the medialis has disappeared behind the trigeminal and from there posteriorly the secondary is the only one present". Now, however, it lies dorsally to the glossopharyngeal nerve, as it has "lapped round" it. At the 11 mm stage, where the cartilage of the skull is well developed, the secondary vein is said to give off a small vein, which accompanies the hyomandibular nerve through the hyomandibular foramen. "From the point of its emergence from the skull there starts a vein which, though in contact with the secondary vein, does not yet communicate with it and ends blindly in front. This vein passes externally to both glossopharyngeal and vagus, and then comes into contact with the secondary vein again behind the vagus. From its relations to the nerves this vein is the vena capitis lateralis." "The secondary vein thus does

[1] In the figure 63 this vein has been put in according to the condition in somewhat younger and older stages.

not here become the lateralis, as it does in Selachians." At a 50 mm stage the lateralis is said to extend "forwards beyond the hyomandibular into the orbital sinus".

These descriptions contain a number of errors or gaps sufficiently serious to obscure the entire question of the vein homologies in ganoid fishes.

At the 8 mm stage of *Amia* the vein system of the head is about as advanced as in the 7 mm stage of DE BEER. Posteriorly the secondary vein "is the only one present" and it has "lapped round" the glossopharyngeal. But there are also three other features of some importance to be noted, viz. 1) that the glossopharyngeal is looped round *ventrally* by a rather wide vein connected at both ends with the "secondary vein", 2) that the first vagus nerve is looped around *dorsally* in a similar way with the posterior cerebral vein attached to this loop, and 3) that there is a short vein branching off from the secondary vein just inside the hyomandibula, this branch being the early rudiment of the so-called "vena capitis lateralis". It extends backward for a short distance.

VOL. 24 1943

The ventral glossopharyngeal loop is a part of the secondary vein proper, and that portion of the jugular vein (secondary vein) which lies dorsally to the glossopharyngeal is a lateralis component, as was shown to be the case in *Squalus* and in *Acipenser*.

The vagus loop corresponds morphologically to the vagus loop in *Acipenser* and to the vagus section of the lateralis in *Squalus*.

The subhyomandibular branch of the secondary vein probably corresponds to the posthyomandibular branches in *Squalus* and *Acipenser* and probably has nothing to do with a "vena capitis lateralis", as present in sharks.

An 11 mm stage (fig. 64) of my collection seems to be in some respects very little more, in others less, advanced than the 11 mm stage reconstructed by DE BEER; therefore it ought to correspond to it fairly closely. Our reconstructions, however, show very little in common. As at the 8 mm stage the head vein has loops around the 1st vagus and the glossopharyngeal nerves. The subhyomandibular branch, very wide inside the hyomandibula, has developed into a considerable system of veins

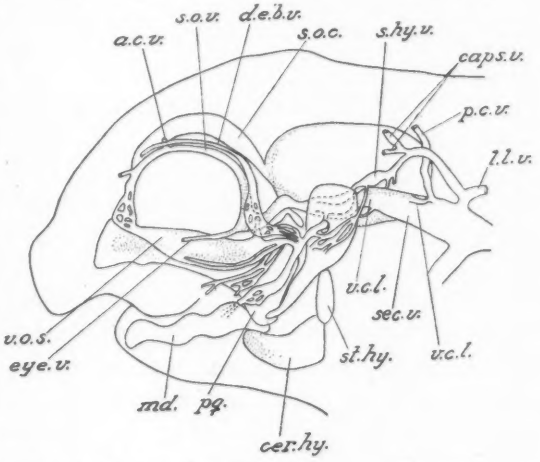


Fig. 64. *Amia calva*. Embryo 11 mm. Venous system of the head.

(the "vena capitis lateralis" of DE BEER). From the anterior end of this system (not from the secondary vein as DE BEER says) there emerges a vein, the hyomandibular, piercing the hyomandibula. Outside the hyomandibula this vein joins a plexus, which is connected with the subhyomandibular vein behind the hyomandibula. The hyomandibular vein, after piercing the hyomandibula, branches into a number of veins, one of which forms a plexus outside the posterior part of the palato-quadrate and inside the mandibula. Another is connected with the posterior end of the lower, and another with the upper, orbital sinus. The subhyomandibular vein bends caudally dorsad, sending off branches on the outside of the auditory capsule. It then turns ventrad to join the secondary vein. At this point a big vein branch is sent caudad to the thymus and to the lateral line. The caudal junction between the subhyomandibular and the secondary vein is secondary, as it is not present in a somewhat younger stage.

Frontally the head vein has, besides the hypophysial vein, 3 branches, one dorsal, one ventral and one lateral. The dorsal is the dorsal orbital vein (the superior orbital sinus of DE BEER) with the anterior cerebral vein. The ventral is a branch to the eye, corresponding perhaps to DE BEER's inferior orbital sinus. The lateral is a short vein joining the big ventral orbital sinus, which includes the inferior orbital vein as well as the inferior orbital sinus.

Although it is not possible to make any detailed comparison between the vein system of the head of *Amia* and that of *Acipenser,* the presence in both cases of the secondary vein as chief vein in the region of the vagus nerve seems to be well established. The subhyomandibular vein system in *Amia* seems to correspond fairly closely to the posthyomandibular vein-branches in *Acipenser,* as corresponding plexa and sinuses are apparently connected in both cases.

The head veins in Lepidosteus platystomus embryos.

The development of the venous system of the head in *Lepidosteus* does not seem to have been studied at all; nevertheless it is not my intention to give any detailed history of its development. I would only point out some interesting features. The reason why I can restrict myself to a minimum of description is the fact that the venous system of the head in *Lepidosteus* is very similar to that in *Amia.* There are, however, differences of some importance, which must be mentioned.

The head vein is "the secondary vein" with a lateralis section dorsally to the glossopharyngeal, just as in *Acipenser* and *Amia.* Vagus and glossopharyngeal loops are present. In a 10.1 mm embryo a subhyomandibular branch forms from the secondary vein inside the hyomandibula. It extends caudolaterad and comes out behind the hyomandibula, where however, it soon ends below the skin. Already in 11 à 12 mm embryos this branch has become detached from the head vein inside the hymandibula, but has grown consider-

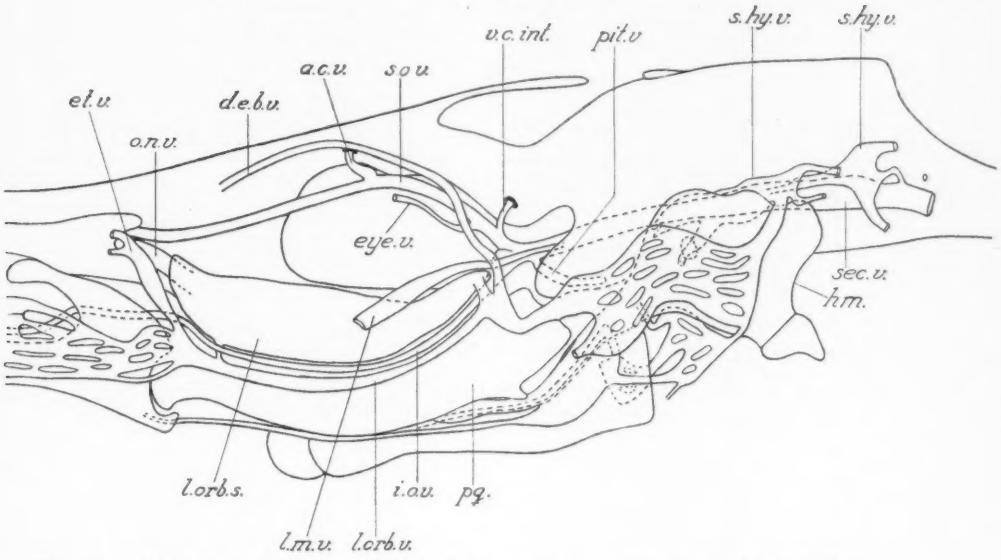


Fig. 65. *Lepidosteus platystomus*. Embryo 33.4 mm. Venous system of the head. Plexa diagrammatically represented.

ably in a caudal direction, giving off branches to the opercular fold and to the auditory capsule. In a 33.4 mm embryo the subhyomandibular vein has become a very considerable and important part of the head-vein system. Already in a 12 mm embryo it has acquired a communication with the secondary vein behind the hyomandibula. The subhyomandibular vein inside the hyomandibula is very wide and saclike. This part at the 33.4 mm stage (fig. 65) sends a branch through the hyomandibular foramen. This branch joins a big plexus on the outside of the hyomandibula, which also has a connection with the head vein behind the hyomandibula. Other branches of the subhyomandibular vein are distributed in the opercular fold and on the auditory capsule. The hindmost branch goes to the thymus and to the lateral line. The extrahyomandibular plexus continues on the outside and inside of the symplectic and the palato-quadrate. It also has a wide communication with the orbital venous system. The communicating vein-branch divides into three vessels, one dorsal in the upper eyebrow, a lower in the lower eyebrow and extending frontad partly to a plexus outside the mandibula, partly to the ethmoidal region. The third vessel is short and joins the saclike lower orbital sinus, which frontally sends a big vein to the ethmoidal region. Frontally the head vein (secondary vein) has 4 branches, viz. the hypophysial, the superior orbital, the inferior orbital and an eye-vein. The superior orbital carries the intermediate cerebral, the anterior cerebral and an eye-vein. The inferior orbital vein runs out frontally inside the mandibula. There is no connection between these veins and the orbital sinus.

VOL. 24 1943

The head veins in Salmo salar embryos.

The head veins of Teleosts have been described by DE BEER in *Salmo trutta* and *Cottus bubalis.*

In 20 mm *Salmo trutta* the vena capitis medialis has since long been absent, except in the trigeminal region, "where it gives off the hypophysial vein". In front the secondary vein runs *ventrally* to the hyomandibular nerve; caudally it is said to run ventrally also to the glossopharyngeal and the vagus. The "vena capitis lateralis" is said to start behind the prefacial commissure of the skull, where the secondary vein exits from the skull and in contact with it. It runs caudad externally to the facialis, glossopharyngeus and vagus, "to reestablish connection with the secondary vein behind the latter nerve". "Both the lateralis and the secondary vein pass internally to the hyomandibula, separated from one another by the hyomandibular muscle, as in *Amia.* The lateralis is similar to that vessel in *Amia,* but the secondary vein differs from that form in that it passes internally instead of externally to the facial. The hypophysial vein runs dorsally to the external rectus muscle, which extends back into the myodome. The oculomotor runs in between two branches of the medialis vein." *Cottus* behaves as *Salmo,* but there is said to be an extra vein present laterally to the hyomandibula, running frontally in "the orbital sinuses" and caudally joining the "lateralis" and the secondary vein in the region of the glossopharyngeus.

This description of *Salmo trutta* by DE BEER does not correspond very closely to the conditions met with in 20 mm *Salmo salar.* 1) the secondary vein in *S. salar* runs ventrally to the vagus, but dorsally to the glossopharyngeus, as in other teleostomes, 2) the "vena capitis lateralis" sends off a small branch trough the hyomandibular foramen, and behind the hyomandibula bends round the edge of it, to continue on its outside frontad as an extra vein corresponding to the extra vein in *Cottus,* 3) the "vena capitis lateralis" is connected frontally with the secondary vein, and the hyomandibular nerve runs in the fork between the two veins, dorsally to the secondary vein but ventrally to the "lateralis". 4) From the point where these two veins are united a third longitudinal vein arises. This vein runs caudad, lying in longitudinal contact with the secondary vein. From it anastomoses issue behind the hyomandibula, in the glossopharyngeal region joining the bent part of the "vena capitis lateralis". The vessel continues caudad, passing outside the glossopharyngeal and also outside the vagus. It has branches to the thymus and to the lateral line (lateral cutaneous vein).

Comparing this with the ganoids, there seems to be not much doubt about the homology between the secondary veins of those fishes, and that in *Salmo,* as in the ganoids, the "vena capitis lateralis" of DE BEER has nothing to do with the vena capitis lateralis in sharks and rays. The subhyomandibular

system of vein branches, however, could not be morphologically compared in detail. As they are connected with vein plexuses and sinuses in similar ways, it is very probable that this system merely represents in different fish somewhat different variations of the same scheme.

The greatest difference between the jugular system of *Salmo* and that of other teleostomians is undoubtedly the relation between the secondary vein and the hyomandibular nerve, the vein lying ventrally to this nerve in *Salmo*, dorsally in the ganoids and, according to DE BEER's reconstruction, also in *Cottus*. This puzzling fact, however, is explained if we investigate 19- and 20-day Salmo (10—12° C.). In embryos of about 19 days and younger the secondary vein lies dorsally to (outside) the hyomandibular nerve. In about 20-day embryos the nerve pierces the vein, i.e. the vein forms a ventral loop around the nerve, and in older embryos the vein lies ventrally to the nerve. The dorsal part of the vein does not quite disappear as a result of this shifting, but remains as a short sac from which the subhyomandibular vein later issues.

VOL. 24 1943

The orbital veins are the hypophysial vein, the upper orbital vein, a large eye-vein, lying as the "inferior orbital sinus" in DE BEER's reconstructions, and a very large lower orbital sinus opening by a short lateral communication into the undivided orbital vein (frontal end of the secondary vein). Caudally this orbital sinus is connected with the "extravein". In embryos about 19 days old the sinus appears as a small vascular sac independent of the rest of the venous system. This sac quickly increases so that already in the 20-day embryo it is a very big sac below the eye. Much later it makes connection with the "extra vein", but as late as in a 20 mm stage it has no other connection.

Summary account of the head veins.

In selachians there are 4 principal systems of veins present arising in the following order, 1) the orbital system, originating from the anterior end of the aortic system, 2) the vena capitis medialis, 3) the secondary vein, developed in the glossopharyngeal and vagus region, below these nerves, and 4) the real vena capitis lateralis, originating in sections dorsally to the vagus, glossopharyngeal and hyomandibular nerves.

In teleostomes the corresponding conditions are as follows: 1) the orbital system, of which, however, the origin is as yet unknown, 2) the vena capitis medialis, 3) the secondary vein, developing below the vagus and glossopharyngeal ganglia and frontally running outside the facialis (*Acipenser*) or the hyomandibularis [other Ganoids and very young Salmo (and Cottus?)] and joining the medialis, 4) the real vena capitis lateralis, present only in short sections, of which one forms a dorsal loop around the 1st vagus nerve and another, in the glossopharyngeal region dorsally to the ganglion, re-

placing a small portion of the secondary vein, which in the grossopharyngeal region is reduced to a ventral loop around the glossopharyngeal (this loop, was not, however, observed in *Salmo*).

In the orbital system a differentiation in orbital veins and orbital sinuses is apparently observable. This differentiation seems to be less advanced in lower forms than in higher. Farthest the development has advanced in *Lepidosteus* and *Salmo*, where the orbital sinuses have become quite independent of the orbital system. The differentiation, however, cannot be said to be gradual in the fishes investigated, though the tendency seems to be perceptible.

The periferal veins to a great extent maintain their connection with the principal system inside or behind the hyomandibula, especially in the region between the facialis and glossopharyngeal nerves.

But the most important result of this investigation of the head veins seems to be that the principal head vein of the Selachians is the vena capitis lateralis, whereas in Teleostomians it is the secondary vein (with the exception of the glossopharyngeal section).

VOL
24
194

THE AUDITORY CAPSULE AND THE GLOSSOPHARYNGEAL NERVE IN SELACHIANS.

In his paper on "The Development of the Skull of Scyllium (Scylliorhinus) canicula L." DE BEER has given an explanation of the relation between the glossopharyngeal nerve and the auditory capsule which does not quite agree with the results of KÄLLBERG's investigations (HOLMGREN, 1940). DE BEER says: "GEGENBAUR (1872) realized that the glossopharyngeal nerve on its passage through the wall of the skull traversed the cavity of the auditory capsule. Since primitively the nerve must have passed behind the capsule, it is of interest to see how the conditions which prevail in Selachians may be explained." It is true that in the Cyclostomes the glossopharyngeal nerve runs posteriorly to the auditory capsule and that one should therefore believe that this should be the primitive condition in vertebrates, as the Cyclostomes are the most primitive of living vertebrates. When considering, however, the question of the primitive relation of the glossopharyngeal nerve to the auditory capsule, it must be borne in mind 1) that the auditory organs in the living Cyclostomes are considerably reduced, 2) that there is no evidence at all for regarding these Cyclostomes as ancestral in relation to the other living fishes, and 3) that in most fishes (except the Cyclostomes) the glossopharyngeal nerve pierces at least part of the auditory capsule.

The first of these statements is of the greatest importance for our question. If it is really true that the auditory organs are reduced in the living Cyclo-

stomes, this condition, might justify the assumption that the glossopharyngeal nerve could behave otherwise in these animals than in the gnathostome fishes. The reduction of the auditory organ in Petromyzonts is evidenced by the fact that the Cephalaspids, so beautifully studied by STENSIÖ, had a normally built auditory organ but with only two semicircular canals. The Pteraspids also had well-developed auditory organ with two semicircular canals. The auditory organ of Myxinoids with but one semicircular canal[1] is apparently also reduced. Thus it seems possible to believe that Petromyzonts as well as Myxinoids were developed from ancestors with well-developed auditory organs.

STENSIÖ (1927) was able to study the nervous system of his Cephalaspids in a way hitherto unknown in the history of palaeontology. He was able to establish *that the glossopharyngeal nerve in these fishes pierces the labyrinth cavity.* STENISÖ says: "As is thus seen from the account given of its course, the n. glossopharyngeus traversed the labyrinth cavity, as it does in several recent fishes with a well-developed labyrinth."

There seems at present to be sufficient reason for demonstrating that it is not *a priori* necessary to believe that the glossopharyngeal nerve primitively ran out posteriorly to the auditory capsule. On the contrary, the evidence supports the assumption that the nerve primitively pierced the auditory capsule and that the conditions in living Cyclostomes are secondary.

On the basis of these points of view, contrary to those expressed by DE BEER, the problem regarding the Selachians becomes reversed. DE BEER's problem was to show that the conditions in Selachians really are the same as in Petromyzonts, i.e. that the glossopharyngeal nerve only apparently pierces the auditory capsule, in reality lying behind it. Supposing that the results of DE BEER are correct and that the nerve in Selachians thus lies posteriorly to the capsule, our problem would be to explain how the nerve came out of the auditory capsule, not how it came to pierce it.

DE BEER's explanation is as follows: "Briefly what has happened is that a lateral shelf from the parachordal cartilages has extended beneath the floor of the auditory capsule. Originally the glossopharyngeal nerve passed out between this shelf (lamina hypotica) beneath and the floor of the capsule above, in a space which represents the fissura metotica, and behind the basivestibular commissure represented in Selachians by the dorsal process of the parachordal plate. Then the floor of the auditory capsule became reduced and fenestrated, while the lamina hypotica persisted and formed a vicarious lower cartilaginous boundary to the capsule. In one place the lamina hypotica does not quite reach the true floor of the capsule, just in front of it, with the result that there is a gap in the cartilage, as may be seen in Text. fig. 19.

[1] This canal probably represents the fused anterior and posterior canals of an auditory organ with two canals.

Behind this point the lateral edge of the lamina hypotica is confluent for a short distance with the lower edge of the outer wall of the capsule, but histological differences in the cartilage mark the limit to which each extends. Further back again, the lamina hypotica extends freely to the side beneath the capsule, which in this region has its true floor chondrified again. The lamina hypotica here forms the lower border of the glossopharyngeal notch. Meanwhile the cavity of the capsule and that portion of the fissura metotica which acts as a glosopharyngeal canal are thrown into one, and the boundary between them is indicated only by some remnants of membrane. At the same time the capsule has bulged backwards to acommodate the large posterior canal, and so it happens that the glossopharyngeal nerve in Selachians seems to penetrate the cavity of the auditory capsule itself, passing in front of the posterior canal, on the hinder side of the lagena, and to run for a short distance close to the posterior branch of the auditory nerve. A minute examination of sections reveals traces of the obliterated fissura metotica, through which both glossopharyngeal and vagus nerves typically pass."

The question of the relation between the glossopharyngeal nerve and the auditory capsule has become more complicated through KÄLLBERG's investigation (HOLMGREN, 1940). As seen from his descriptions, there are considerable differences in the development of the auditory capsule in the different selachians studied by him. Two extremes are present, the one represented by *Squalus, Etmopterus* and *Heterodontus,* the other by *Raja.* Intermediary are *Scyllium, Torpedo* and *Urolophus.*

We begin with *Etmopterus* as representing the first group. In the descriptions of the developmental stages (HOLMGREN, 1940) it has been demonstrated that the rudiment of the auditory capsule consists of an anterior auditory cartilage connected with the parachordals through the anterior basicapsular commissure and a posterior auditory cartilage at the 25 mm stage, independent, but later fusing with a posterior basicapsular commissure. In addition to these structures the processus dorsalis (rudiment of the medial wall of the auditory capsule) is developed anteriorly to the point where the glossopharyngeal nerve leaves the brain cavity. At this stage the glossopharyngeal nerve enters the auditory capsule rudiment, passes below the posterior semicircular canal and runs dorsally to the posterior auditory cartilage rudiment, leaving the auditory region at the caudo-lateral border of this rudiment. Thus there could be no doubt about the primary crossing of the nerve over the floor of the auditory capsule in *Etmopterus.*

But at the next stage of development (stage 30 mm) matters have grown somewhat more complicated through the development of the cartilage of the posterior semicircular canal of KÄLLBERG. This cartilage partly forms a portion of the inner wall of the auditory capsule behind the glossopharyngeal root, partly a lamella on the ventral and posterior side of the posterior

VOL
24
194

semicircular canal, forming a gutter enclosing this canal. The glossopharyngeal nerve runs, as at the preceding stage, dorsally to the posterior auditory commissure but ventrally to the tip of KÄLLBERG's cartilage. The relation between the glossopharyngeal nerve and the posterior auditory capsule cartilage has undergone no change through the development of KÄLLBERG's cartilage.

As in the following development (fig. 66) KÄLLBERG's cartilage forms the posterior pole of the auditory capsule, this has no influence upon the course of the glossopharyngeal nerve, which comes out from the auditory capsule between KÄLLBERG's cartilage and the posterior auditory cartilage. The primary auditory capsule in *Etmopterus* is thus open posteriorly, owing to the fact that the posterior auditory cartilage bends up only laterally, not posteriorly. Its function of forming the posterior wall of the capsule has been taken over vicariously by a part of KÄLLBERG's cartilage.

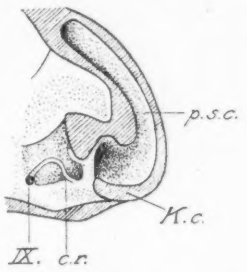


Fig. 66. *Etmopterus spinax*. Embryo 43 mm. Reconstruction of the medial wall of the posterior part of the auditory capsule.

The development of the auditory capsule in *Etmopterus* shows that there is no reason for *not* concluding that the glossopharyngeal nerve penetrates the auditory capsule in *Etmopterus*. The development of the auditory capsule in *Squalus* essentially follows the same lines as in *Etmopterus*. As the auditory capsule at certain young stages of *Heterodontus* is the same as in corresponding stages in *Etmopterus* and *Squalus*, *Heterodontus* probably also belongs to the same group as these two genera in regard to the development of the auditory capsule.

In *Raja* the conditions are different. The description given in the first part of this work (HOLMGREN, 1940) of the developmental stages of the auditory capsule in *Raja* is very interesting, as it shows that the inner wall of the auditory capsule upon its first appearance forms a continuum, divided into an anterior and a posterior portion by the glossopharyngeal nerve. The floor of the capsule is then formed from the rudiments of the two basicapsular commissures, and the auditory capsule proper from two independent blastematic rudiments (stage 40 mm). These latter (stage 43 mm) fuse with the basicapsular commissures. The posterior capsular rudiment has grown out in a caudal and a lateral direction and has become considerably enlarged. The glossopharyngeal nerve passes through a notch in the inner wall of the auditory capsule into the future capsule, where it runs dorsally to the posterior capsular rudiment. Thus at the 43 mm stage the nerve undoubtedly runs through the posterior part of the auditory capsule.

At the 46 mm stage the floor of the auditory capsule is completed. The basicapsular commissures have become fused with the auditory cartilages and these latter have also fused. A small basicapsular fenestra is present. The

auditory cartilages have bent up on the sides of the auditory organ. The anterior and posterior borders of the capsule have likewise bent up. The posterior border of the posterior capsular rudiment has grown upwards, thus enclosing the glossopharyngeal nerve in a foramen at the posterolateral corner of the capsular rudiment. There is no trace of any cartilaginous parts enclosing the posterior semicircular canal. The medial wall of the auditory capsule has now grown up enclosing the root of the glossopharyngeal nerve in a foramen, as the anterior and posterior part of this wall have fused dorsally to the nerve. Thus the glossopharyngeal nerve passes through the auditory capsule and leaves it through a foramen of its own, piercing the posterior wall of the capsule, which is built up of the posterior auditory cartilage rudiment. In *Raja* the cartilage of KÄLLBERG is represented by the posterior (postglossopharyngeal) part of the medial wall of the capsule.

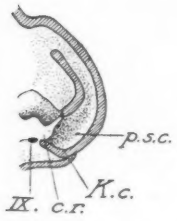


Fig. 67. *Scyllium canicula*. Embryo 38 mm. Reconstruction of the medial wall of the posterior part of the auditory capsule.

At later embryonic stages of *Raja* the conditions do not alter.

From the description of the development of the auditory capsule of *Scyllium* (HOLMGREN, 1940) it is evident that in principle *Scyllium* behaves like *Etmopterus*. The only difference is found in the development of KÄLLBERG's cartilage, which in *Scyllium* (fig. 67) quantitatively plays a minor part in the formation of the posterior pole of the auditory capsule. The lateral capsular wall of the anterior part of the posterior semicircular canal is formed by a longitudinal cartilaginous rod extending caudad from the posterodorsal border of the glossopharyngeal foramen to the roof cartilage of the posterior semicircular canal. The capsular canal of the posterior semicircular canal in this part is incomplete and the glossopharyngeal nerve runs together with the posterior auditory nerve branch close to the ventrolateral border of the semicircular canal.

The conditions in all young stages of development of the auditory capsule in *Scyllium* make it evident that the glossopharyngeal nerve penetrates the primary auditory capsule, as explained before regarding this nerve in *Etmopterus* and *Raja*. The part of the auditory capsule interpreted by DE BEER as being the primary auditory capsule is a secondary posterior part of it, developed from the medial wall of the capsule as the cartilage of KÄLLBERG. That "lateral shelf from the parachordal cartilages" (or "lamina hypotica") is the posterior basicapsular commissure of which the posterior margin extends backwards below the glossopharyngeal nerve. In rays part of the pharyngohyal is added to this margin, as explained earlier. There is no evidence from the course of development that this shelf is an independent lamina below the "primary auditory capsule", as it is really a part of the

capsule proper. The gap in the cartilage described by DE BEER is the fenestra basicranialis, and the remnants of membrane which would merely separate the primary capsule from the fissura metotica, could not be found in any of our sections. At all our stages the fissura metotica has nothing to do with the glossopharyngeal nerve, as this nerve enters the auditory capsule through a foramen in its medial wall far in front of the fissura metotica. The early development of *Raja* demonstrates that the fissura metotica is primarily the incisure between the occipital arch and the posterior part of the processus dorsalis, and that this incisure might possibly be serially homologous with the incisure between the anterior and the posterior part of the processus dorsalis through which the glossopharyngeus passes. Theoretically and strictly morphologically a widening of the fissura metotica frontally could never cause this fissure and the fenestra basicapsularis to combine into one. The fissura metotica belongs to the lateral wall of the cranial cavity, the fenestra basicranialis to the floor of the auditory capsule. The real fissura metotica can only move along the more or less vertical lateral wall of the cranial cavity, the fenestra basicranialis only along the more or less horizontal floor of the auditory capsule. But this is perhaps a too formal point of view.

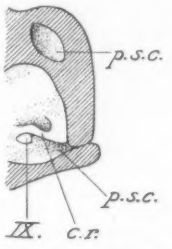


Fig. 68. *Urolophus Halleri.* Embryo 49 mm. Reconstruction of the medial wall of the posterior part of the auditory capsule.

In *Torpedo* the conditions are intermediate between those in *Scyllium* and *Raja*. As in *Raja*, no independent cartilage of KÄLLBERG is formed, but the postglossopharyngeal part of the medial wall of the auditory capsule is formed in continuity with the parachordal plate, as is the preglossopharyngeal. The posterior part of the medial wall of the auditory capsule in *Torpedo* throws out a lamella, which forms a roof above the anterior part of the ventral portion of the posterior semicircular canal. Laterally, this roof bends down laterally to the canal and in a very short section joins the medial wall ventrally to the canal at the dorsal border of the glossopharyngeal foramen[1]. The lateral wall of the canal ceases caudally with this ventral fusion. Thus the part of the medial wall corresponding to KÄLLBERG's cartilage in *Torpedo* plays very little part in the formation of the interior skeleton of the auditory capsule. It is, however, of some interest to state that structures corresponding to parts of the cartilage of KÄLLBERG of sharks are likewise present in a ray.

In *Urolophus* (fig. 68) the conditions are almost exactly the same as in *Scyllium*, except that the cartilaginous roof of the posterior semicircular canal

[1] The very short connecting piece corresponds to the rodlike cartilage process in *Scyllium*.

soon ceases posteriorly. The medial wall of the auditory capsule or dependent outgrowths from it plays no part in the formation of the posterior pole of the auditory capsule.

Before attempting a phylogenetical explanation of the conditions of the region of the posterior semicircular canal, we shall now consider briefly the conditions in *Heterodontus*. As already stated, in principle they are the same as in *Etmopterus* and *Squalus*. There is a cartilage of KÄLLBERG present forming the posterior part of the medial wall of the auditory capsule. This cartilage also throws out a lamella, which bends round the ventral side of the posterior semicircular canal to meet caudally a similar lamella which forms the roof of the same canal. That part of the lateral border of the ventral lamella which will fuse more posteriorly with the roof lamella thickens, whereas the ventral part of it below the semicircular canal is very thin. The thickened border of the lamella runs laterally to the semicircular canal and corresponds in every respect to the cartilaginous rod present in *Scyllium*, as well as in *Urolophus*, and to a small extent also in *Torpedo*. In reality, the conditions of *Scyllium* and *Urolophus* would be attained if the thinned part of the cartilaginous wall of the semicircular canal mentioned above were to disappear and the canal were to open ventrally. A difference, however, occurs at a more caudal point where, in the sharks, the cartilage of KÄLLBERG or the medial wall of the auditory capsule forms at least part of the posterior pole of the auditory capsule, whereas in the rays the medial wall has very little to do with this formation, this part of the capsule being formed by the posterior auditory cartilage and the posterior basicapsular commissure.

A comparison between the reconstructions of the posterior part of the auditory capsule of *Scyllium* and that of *Urolophus* makes it evident that the cartilage of KÄLLBERG and the posterior part of the medial wall of the auditory capsule are homologous structures. The difference is that in *Scyllium* the cartilage of KÄLLBERG sends out a lamella covering the lower part of the posterior semicircular canal on its lower side, whereas in *Urolophus* no such lamella is developed. Owing to the existence of this lamella in *Scyllium* the wall of the auditory capsule below the posterior semicircular canal is double and the glossopharyngeal nerve passes between the bottoms. The lower floor is the posterior basicapsular commissure and the basiotic plate. In *Urolophus* the floor is single and the glossopharyngeal nerve and the posterior canal lie in the same cavity. The floor in *Urolophus* corresponds to the lower bottom in *Scyllium*.

Etmopterus, *Squalus* and *Heterodontus* all behave in the same way as *Scyllium*, since they all have a double floor below the posterior semicircular canal. Like *Urolophus*, both *Torpedo* and *Raja* have a single floor. *Torpedo* is reminiscent of *Urolophus* and the sharks as there is a rodlike bridge round

the posterior semicircular canal at the level of the inner glossopharyngeal foramen. This bridge, however, is very slender. In *Raja* it is lacking and the posterior semicircular canal lies uncovered in the auditory capsule cavity.

Now the question arises: Which of these conditions is to be considered the primary one? The *Raja*-like or the *Etmopterus*-like or perhaps the *Urolophus*-like condition? As the sharks all have the same complicated structures, it must be regarded as more or less probable that they have evolved them from earlier sharks types with an independently chondrifying posterior medial wall of the auditory capsule. This independence could scarcely be taken for primary as the sharks are the only fishes with such independence. We must imagine that this independence is due to the fact that the medial wall of the auditory capsule has acquired greater dimensions and significance in the sharks than in other selachians and therefore possesses a special centre of chondrification, which is lacking in those types in which this part of the auditory capsule is only of importance as a posterior part of the medial wall of the capsule. I suggest that the postglossopharyngeal part of the medial wall developed originally like the preglossopharyngeal, viz. as a processus dorsalis growing out from the parachordal plate. As the former part overtook, in sharks, the forming of the posterior pole of the capsule, it acquired a special centre of chondrification, which did not arise in the rays, in which the posterior pole is essentially formed from the posterior auditory cartilage and the posterior basicapsular commissure. Reasoning in this way we come to the result that in regard to the mode of forming the medial wall of the auditory capsule the rays are more primitive than the sharks. This result, however, must not be taken for granted, as it rests upon an assumption, which may possibly be erroneous.

Assuming the preceding reasoning to be correct, it would be possible to find another primary quality of the posterior part of the medial wall. In *Torpedo* and *Urolophus* the posterior semicircular canal is enclosed for a short distance in a very short cartilaginous canal formed out of the medial wall. A part corresponding to this canal is present in the sharks. We are therefore justified in believing that a corresponding structure was also present in the predecessors of sharks and rays. In *Urolophus* the said structure is developed essentially as in *Scyllium*, in *Torpedo* it is much more reduced and in *Raja* it is lacking altogether. The *Urolophus* and *Torpedo* conditions are thus more closely related to those in the sharks than those of *Raja*. If I were to try to form some idea of the ancestral type of the medial wall of the auditory capsule in selachians I should describe this type in conformity with the conditions in *Urolophus* and *Torpedo*. The posterior part of the medial wall in the ancestral type must have developed from the parachordal plate and did not form any considerable part of the posterior part of the auditory capsule and was provided with a cartilaginous bridge enclosing the

VOL. 24 1943

lower part of the posterior semicircular canal. The floor of the posterior part of the auditory capsule was single.

Raja differs from the other rays, as there the bridge is absent. This condition might be a consequence of the quite different aspect of its membranous auditory labyrinth organs in the embryos. In the other rays the labyrinth organs are similar to those in sharks, with well separated parts in which the semicircular canals are narrow, whereas the different parts in the embryonal *Raja* labyrinth lie close together pressed against one another. In addition, the canals are very wide, with thin walls. There is very little mesenchymatic tissue between the different parts. Only the lateral semicircular canal is bridged over by cartilage. It is possible that this special character of the auditory organs in *Raja* has something to do with the simplicity of its auditory capsule. This simplicity might have arisen through reduction from the *Urolophus* or *Torpedo* conditions.

Whether these phylogenetical speculations are right or wrong, one thing is certain: the glossopharyngeal nerve penetrates the auditory capsule. Thus at young stages at least no special "lamina hypotica" is present, this structure being part of the posterior basicapsular commissure.

In *Chimaera* the glossopharyngeal nerve behaves as in sharks and rays. In spite of DE BEER's statement (1935) to the contrary, the *nerve traverses the auditory capsule*. The medial wall of the capsule is a very thin membrane, and the sections through the auditory region of a young *Chimaera* show with the greatest clearness the nerve lying laterally to this membrane.

THE POSTERIOR MYODOME.

Although the comparative anatomy of the posterior myodome has been the subject of numerous investigations, I cannot say that its problems have been satisfactorily solved. This is due in some measure to the inadequate interpretation of certain adjacent structures. ALLIS (1914) has pointed out that the so-called lateral commissure of the neural skull which constitutes the lateral wall of the trigemino-facialis chamber is of visceral origin, whereas DE BEER opposes this view. As the lateral commissure plays a fundamental rôle in the formation of the dorsal compartment of the myodome, it seems very necessary to have the question of the lateral commissure settled before the myodome problem can be taken up in earnest. Another question that also must be answered is that of the parachordals in ganoids and bony fishes, which has not yet been satisfactorily answered. A third question of the greatest importance in regard to the myodome is that of the relation between the trabeculae and polar cartilages and the lateral walls of the brain-case in *Amia* and teleosts.

STUDIES ON THE HEAD OF FISHES

1. *The lateral commissure.*

According to ALLIS' theory, the lateral wall of the trigemino-facialis chamber (the lateral commissure of DE BEER) represents the otic process of the palato-quadrate in *Ceratodus*. "The processus oticus thus being represented, when present, in *Amia*, teleosts and selachians, in a part of the cranial wall of those fishes, it is evident that the processus metapterygoideus of *Amia*, and its homologue in teleosts and selachians (ALLIS, 1914 a) cannot be a processus oticus, as I and most other authors have heretofore considered it, and as the relations of this process, in *Amia*, teleosts and *Heptanchus*, to the nerves and vessels of the region do not permit of its being considered as a processus basalis, it would seem as if it must be considered as a special process developed in relation to, or in connection with, the musculus levator arcus palatini. In *Ceratodus* and *Chimaera* this process is apparently wholly wanting, as is also the M. levator arcus palatini; in *Chlamydoselachus* it is but slightly developed in *Heptanchus* it is largely developed...".

VOL. 24 1943

According to DE BEER (1926), the lateral commissure is an extension of the subocular shelf, developing in front or behind the palatine nerve, or both in front and behind. "In front it may represent an extended basitrabecular process; behind, it must be an extended post-palatine commissure."

According to ALLIS, the lateral commissure is thus a visceral structure fused on to the neurocranium; according to DE BEER it is a neurocranial structure.

DE BEER has devoted a chapter of criticism to ALLIS' theory. This criticism falls under the following headings: "1) the confusion of cranial and visceral structures: 2) the impossibility of the theory when applied to Selachii; 3) the true nature of the lateral commissure and pila lateralis as evidenced by their development and the relations of the palatine nerve."

The first two of these objections are rejected by my description of the development of the lateral commissure in sharks and rays (HOLMGREN, 1940), where it was clearly stated that the lateral commissure begins its development as a process of the palato-quadrate, which liberates itself from the palato-quadrate and fuses on to the neural skull, there forming the lateral wall of the trigemino-facialis chamber. In the preceding pages I have also furnished evidence of a similar development in *Amia* and teleosts. The lateral commissure really *is* a visceral structure. ALLIS has compared the commissure with the processus oticus of *Ceratodus*.[1] This is a correct comparison. "But now, according to him (ALLIS), the otic process in the Selachians would be represented by the cartilage which in *Squalus* encloses the orbital artery and the head vein, and not by the process of the pterygo-quadrate. This means

[1] Compare HUXLEY (1876). HUXLEY apparently considers the spiracular cartilages as a part of the otic process. (Compare HOLMGREN, 1940).

rejecting a structure with all the necessary relations and prefering to it another with only some of the relations of the otic process of higher forms. There is no further evidence to warrant this, in my opinion, inadmissible and disadvantageous exchange." (DE BEER, 1926). In the preceding pages I have given evidence for the occurrence in Selachians of two processus otici, a *processus oticus internus* and a *processus oticus externus*. The former is the lateral commissure in Selachians, Ganoids and Teleosts and the processus oticus in Dipnoi (*Ceratodus, Protopterus* and *Lepidosiren*), the latter is the processus oticus in Notidanids, and perhaps the anterior process of the metapterygoid process (ALLIS, 1914) in Ganoids and Teleosts. In the lines quoted (p. 149) ALLIS has already suggested this solution of the problem.

Concerning the third objection of DE BEER it may simply be remarked that an outgrowth of the subocular shelf and of the post-palatine commissure of the neurocranium is not the only method by which the palatine nerve could be enclosed in a canal in the lateral commissure. The growth of a visceral lateral commissure might also have a similar effect.

To aid us in solving the myodome question therefore it is of great importance to remember that the lateral commissure *is* an extracranial structure plastered on to the primary cranial wall.

2. *The parachordal question in Holostei and Teleosts.*

In Selachians the parachordals are always formed in close contact with the notochord, where they form bandlike, frontally attenuated lamellae jutting out on either side of it. Immediately outside each parachordal lies the lamina basiotica, of which the anterior part forms a more or less developed preotic portion of the lamina basiotica (especially pronounced in rays). (HOLMGREN, 1940). The basicapsular commissures are attached to the otic portion of this lamina.

In *Holostei* and Teleosts the parachordals are described as cartilaginous rods frontally separated from the notochord by a broad gap. Caudally the rods converge and reach the notochord in the posterior part of the auditory region. The basicapsular commissures are directly attached to the lateral margin of the "parachordals". VAN WIJHE (1922) has already suggested that the "parachordals" of *Amia* are not the true parachordals. If we compare the two descriptions just given, it is obvious that the description of the "parachordalia" of the teleostomians tallies much better with that of the lamina basiotica of the Selachians than with their parachordals. We may ask then: where are the parachordals of the *Amia* and Teleosts? According to STÖHR (1882), SWINNERTON (1902) and PEHRSON (1922) the parachordals appear in two portions: posterior and anterior parachordal plates. The former are confined to the vagal portion of the skull. The latter are said to appear as

a separate anterior cartilaginous centre, which extends caudad, gradually nearing the notochord and joining the posterior parachordals. Other authors cannot confirm the findings of STÖHR concerning the two centres, but believe the entire cartilaginous rod to be a single piece. As the morphology of the parachordals has some importance for our study of the myodome question, I have studied the ontogeny of these cranial elements in *Salmo salar*. In an embryo 55 days and 6 hours old (reared at c:a 4° C) posterior parachordals are tolerably well demarcated in the postvagal region of the notochord (fig. 14). They are prochondrial. They lie close to the notochord, diverging frontally from the latter and reaching a separate prochondrial rod, running frontad at the ventromedial border of the auditory vesicle. This rod is apparently STÖHR's anterior parachordal plate. If compared with Selachians, however, the rod must be considered to be the basiotic plate, which has the same relation to the auditory vesicle and which develops laterally to the true parachordals which are there present. In very young embryos of *Salmo salar* the space between the notochord and this basiotic plate is filled in with an undifferentiated mesenchyme with scattered cells. At a stage of development, 83 days old, the mesenchyme in the gap between the basiotic plate and the notochord is somewhat differentiated as its cell-nuclei are larger and the intercellular substance assumes, in sections stained with Azan-Malory mixture, a light-bluish colour, so that a somewhat differentiated thick lamella has arisen in the gap. At later stages this lamella gradually chondrifies. During this development the thin auditory capsules have arisen in connection with the basiotic laminae, which now appear as thick medial borders of the capsules. To begin with, the chondrifying lamella is thick, connecting the entire lateral surface of the notochord with the entire medial border of the basiotic lamina, but later, as the chondrification advances, the lamina becomes thinner and located to the upper half of the space between the notochord and the lamina basiotica. There is thus formed a longitudinal medial groove ventrally between the two basiotic laminae, a groove of which the bottom is formed by the notochord and the described spacefilling laminae and the border of the basiotic laminae. The space-filling laminae must be considered to be *the true parachordals*. As in the course of development the laminae basicapsulares approach the notochord, the true parachordals gradually diminish their lateral extension and are finally reduced to a narrow strip of cartilage between the notochord and the basiotic lamina. Eventually this strip is completely fused with the basiotic lamina and is no longer distinguishable. An exception from this process of development is the most frontal part of the parachordals, which does not narrow down like the rest of them and which does not chondrify until a late stage. This part forms the dorsal surface of the rudiment of the prootic bridge.

Thus the floor of the brain-case of the chordal part of the skull is prac-

tically made up of the laminae basiotici and the anterior (prootic bridge) and posterior (occipital) parts of the parachordals. In the course of development the anterior end of the border of the basiotic lamina extends as a process frontad to meet the caudal end of the trabecular system. This part seems to be comparable with the preotic part of the basiotic lamina in Selachians (HOLMGREN, 1940).

3. *The relation of the lateral wall of the brain-case and the trabecular system in Amia, Lepidosteus and Salmo.*

In an *Amia* embryo about 11 mm long the brain-case is completely closed, partly by means of cartilage, partly by membrane (fig. 69). In the orbito-temporal region the supraorbital cartilage and the trabeculae are chondrified, but all other parts are membranous. In the auditory region the otic capsules and the lateral commissure are chondrified or in process of chondrifying, whereas the medial wall of the trigemino-facialis chamber is membranous. It is a noteworthy fact that the membranous lateral walls of the brain-case do not join the lateral edges of the trabecular system but are attached more or less medially to this border, so that the trabeculae and polar cartilages form a rather broad shelf on either side of the basis cranii. In the region of the ethmoidal plate (trabecula communis) the membranous side walls of the brain-case merge ventrally into a broad and thick horizontal lamella, which covers the medial two-fourths of the plate. This lamella may be described as a broad and low interorbital septum (i.o.s.). Where the ethmoidal plate divides caudally into the two paired trabeculae, the latter are joined together by a connective tissue lamina. The side walls of the brain-case of this region join this connecting lamina, the trabeculae taking no part in the formation of the brain-case. In the region of the polar cartilage the membranous lateral wall of the brain-case adheres closely to the mediodorsal surface of the polar cartilage, forming a covering on this surface, more medially connecting up

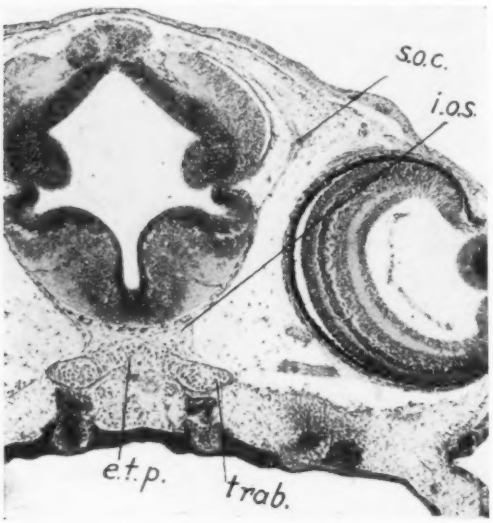


Fig. 69. *Amia calva.* Embryo 10.6 mm. Transverse section through the interorbital septum in front of the optic nerve.

with the membrane which lies below the hypophysis. It has no connection with the lateral commissure already well chondrified at this stage. On a level with the hypophysis the membranous wall again becomes detached from the cartilaginous ventral parts and joins the connecting tissue in which the hypophysis is sunk and which transmits the hypophysial vein. The polar cartilages connect up caudally without any boundary line, with the anterior ends of the laminae basioticae.

It is obvious from this description that there is in *Amia* a deep triangular longitudinal groove entering from each lateral side between the trabecular system and the wall of the brain-case. The deepest parts of these two grooves medially are broadly separated. This condition may also be described thus: there is a broad but very low interorbital septum present in *Amia*.

Lepidosteus behaves in much the same way as *Amia*, but with the following exceptions. In the region of the ethmoidal plate the side wall of the brain-case connects with the dorso-lateral corner of that plate. In the region of the paired trabeculae and the polar cartilages the side wall connects with the dorso-mesial surface of these cartilages. The lateral groove between the trabecula and the side wall is therefore less pronounced than in *Amia*, and the subocular shelf narrow. Only in the posterior part of the hypophysial region does a deep pocket form between the polar cartilage (or anterior end of the lamina basiotica) and the side wall.

(In *Polypterus* (45 mm) the side wall of the brain-case rises from the lateral border of the trabecula, there being thus no subocular shelf.)

In *Salmo* the conditions are principally as in *Amia* but with some important differences, due especially to the development of a high interorbital septum.

The membranous lateral wall of the brain-case of the anterior orbito-temporal region connects medio-ventrally with the interorbital septum, which ventrally joins the ethmoidal plate at its medial line. In a 17 mm embryo the septum is comparatively thick and low in this region, but further back it grows thinner and higher. Where the ethmoidal plate merges caudally into the paired trabeculae, the septum becomes lower and broader and forms a cell mass connecting the medial parts of the trabeculae with one another. This cell mass is connected dorsolaterally with the membranous lateral wall of the brain-case. This connecting cell mass may be followed below the hypophysis as a quadrangular plate from the dorsal corners of which a membrane turns dorsad, forming the lateral walls of a pocket in which the hypophysis is lodged. Towards the posterior end of the hypophysis this side wall sends out a medial lamella from its dorsal border. This is the lamella which enters between the hypophysis and the hypothalmus and represents the frontal medial part of the membranous prootic bridge. The lateral wall of the brain-case is connected along the line of junction between the lateral wall of the hypophysial sac and the membranous prootic bridge. Ventro-laterally this wall

VOL. 24 1943

passes close to the ventral part of the lateral commissure and fuses with it somewhat later. In a simplified way the long description, which is hard to understand, may be summarized as follows: The interorbital septum grows thicker and lower towards the hypophysial region; there the hypophysis is sunk deep down in the upper part of this septum. Behind the hypophysis the septum continues backwards, extending between the floor of the brain-case—the prootic bridge—and the laminae basioticae. This part of the "septum" ends at the tip of the notochord. As the ventral end of the septum is attached at the medial parts of the trabeculae, the polar cartilages and laminae basioticae, there arises on each side a more or less deep, in the temporal region triangular, space between the lateral wall of the brain-case and the latter structures, which form a subocular shelf. This space deepens to a pocket in the region of the posterior part of the hypophysis. At later stages of development this pocket deepens considerably and extends along the posthypophysial septum to the tip of the notochord, passing beneath the prootic bridge.

The preceding paragraphs were intended to give some facta that are necessary for an understanding of the myodome question. In a following paragraph I shall proceed to a description of the later development of the myodome of *Salmo salar.*

4. *Theories on the morphological nature of the posterior myodome.*

According to ALLIS (1919) the posterior myodome of teleosts generally consists of a dorsal compartment containing the m. rectus externus, and a ventral compartment containing the other rectus muscles. The two compartments are separated through the horizontal myodomic septum. In addition each compartment is divided by means of a medial (vertical) myodomic septum into a right and a left half.

In his work on Mail-cheeked fishes ALLIS (1909) came to the conclusion that the myodome "was primarily a subpituitary and intramural space which had been secondarily invaded by certain of the rectus muscles, entrance to it having been acquired, on either side, through a foramen that transmitted a cross-commissural vein which drained the pituitary region and more particularly the hypophysis". (ALLIS, 1919). In coming to this conclusion ALLIS thus did not accept SWINNERTON's opinion that the anterior portion of the myodome was derived from the cavum cranii, a contention that was supported by GAUPP (1906).

According to GAUPP (1906) the myodome of *Salmo* may be divided into three parts. The floor of the myodome is made up of the trabeculae and the membrane connecting them; the roof consists of the membranous floor of the cranial cavity, and the lateral boundary is the "Seitenwandknorpel".

Caudally, this part is followed by the medium part of the myodome, and ventrally it opens into the orbit. The medium part is said to be located between the anterior parachordalia (laminae basicapsulares) and the brain, thus dorsally to the primordial basis cranii, forming a part of the cavum cranii. The posterior myodomic part arises ventrally to the basis cranii and dorsally to the parasphenoid. This description of GAUPP thus contains the statement that the anterior part of the myodome is extracranial; the medium part intracranial and the posterior extracranial.

In his paper of 1919 ALLIS has given a very exhaustive analysis of the myodome in different fishes and tetrapods. Concerning the myodome in non-siluroid Teleostei, ALLIS says: "The myodome is always separated from the cavum cerebrale cranii by membrane (dura mater), cartilage or bone, and the separating wall is in part spinal and in part prespinal in position". From this statement it seems to follow that the myodome is extracranial all through. The dorsal compartment in most Teleostei "is always more or less prolonged posteriorly into the basioccipital region and the ventral compartment is also frequently so prolonged". "The posterior part of the basioccipital portion of the myodome lies between the ventrolateral vertebral processes which are quite certainly the homologues of the haemal arches of the tail". These conditions "thus lead inevitably to the assumption that the entire dorsal myodomic cavity is a subvertebral canal similar to the haemal canal in the tail" ... The hypophysis and the pituitary veins became lodged in its anterior portion. The external rectus muscles of the eye then invaded this canal, whereas the other rectus muscles retained their insertion into the external surface of the preclinoid wall (*Amia*). Then the condition in most Teleosts arose through resorption of the preclinoid wall, the pedicel of the alisphenoid and such parts of the basicapsular commissures as constitute the lateral walls of the subpituitary portion of the myodome. "Because of the resorption of the preclinoid wall and its replacement by membrane, the musculi recti interni ... have first sought firmer attachement on the dorsal surface of the persisting portions of the canales parabasales. The membranes resulting from the resorption of the preclinoid wall were then pressed together in the median line by the recti interni and form a median vertical myodomic membrane".

From ALLIS' description it would seem that he considered the myodome of most bony fishes to be an extracranial structure.

DE BEER'S (1926) interpretation of the myodome is very different. SCHLEIP (1904)[1] and DE BEER recognize the three following portions: "(1) The anterior portion of the myodome is dorsal to the trabeculae and polar cartilages. Its sides are formed by the lateral commissure. In *Amia* it is in communication with the trigemino-facialis chamber. This portion of the

[1] GAUPP (1906) and ALLIS (1919) also distinguished three portions, all slightly different in the papers of the different authors.

myodome is ventral to the dura mater, between it and the cartilaginous floor of the skull. If the dura mater is regarded as marking the position of the innermost membranous wall of the skull, this portion of the myodome can be called intramural. From the present point of view, however, it is dorsal to the skull-floor and therefore intracranial."

(2) "The middle portion begins beneath the anterior edge of the prootic bridge and extends back as far as to the beginning of the parachordals (i.e. the edge of the basicranial fenestra). Its roof is the prootic bridge, its sides are the polar cartilages; it has strictly no cartilaginous floor since it lies in the hindmost portion of the hypophysial fenestra. It is therefore intramural."

(3) "The posterior portion of the myodome begins beneath the anterior edge of the parachordals and extends backwards. Its sides are ventral processes of the parachordals, which continue on a level with the inner margins of the hind ends of the polar cartilages. This portion is therefore extracranial."

There are thus at least three different opinions about the myodome. In order to arrive at an understanding of the myodome I have undertaken an examination of the embryological development of the interorbital septum and the myodome in *Salmo salar*. The results of this investigation now follow.

VOL
24
194

5. *The ontogenetical development of the interorbital septum and the myodome in Salmo salar.*

Stage, 41 days old (reared in tapwater, 10—12° C).

No membranous cranial wall has as yet developed, the brain being surrounded only by an indifferent mesenchyme, without any boundary merging into the general mesenchyme of the head. In the ventral parts, especially behind the optic nerve, the nuclei of the mesenchymatic cells and the mesenchymatic fibres are arranged more or less parallel with one another and with the surface of the brain. A similar arrangement is also discernible in front of the opticus. An interorbital septum is not defined, although its later appearance is foreshadowed by the mesenchyme which covers the brain ventrally.

The trabecular commissure is forming. It arises from a medial, longitudinal band of densely accumulated cells located below the level of the trabecles. From this band cells are seen entering dorsally between the trabecles. The bulk of these cells remain between the trabecles, where they form the rudiment of the trabecular commissure. A number of the cells, however, emigrate dorsad between the trabecles and reach a level dorsally to the trabecles, there forming a medial, longitudinal band of cells. Subsequently these cells take part in the formation of the interorbital septum. In the part behind the trabecular commissure the mesenchymatic band lies between the two trabecles and has a deep medial portion dorsally to the level of the trabecles. Behind the hypothalmus and the hypophysis there follows a mesenchymatic

mass which fills up the plica encephali ventralis. This mass probably corresponds to the acrochordal tissue in sharks (HOLMGREN, 1940) and rays. In the area on a level with the hypophysis the ventral and lateral mesenchyme surrounding the hypothalmus is compressed between the brain and the anterior group of the eye muscles (rectus superior, inferior and internus). From this condition there results a sort of membranous cranial wall. A similar compression takes place between the hypophysis and the intertrabecular mesenchyme. Where the hypophysis is still connected with the epithelium of the mouth cavity the intertrabecular cell mass is penetrated by the hypophysial stalk, behind which the intertrabecular cell mass becomes less distinct. The musculus rectus externus runs in front of the still mesenchymatic or prochondrial lateral commissure and inserts itself near the tip of the notochord probably in the parachordal tissue. (It is also possible, however, that the tissue in question belongs to the acrochordal cell mass.) In relation to those parts of the cranial wall which are represented at this stage of development, the eye muscles are located completely extracranially.

VOL. 24 1943

Stage, 44 days old.

This stage does not differ very much from the preceding. The cranial wall of the trabecular region is only suggested by the tangential arrangement of the mesenchymatic fibres (fig. 70) and the cell nuclei in relation to the brain. The interorbital septum is low and not yet well delimited, but the cell material of which it is forming is more densely nucleated than the surrounding mesenchyme. Ventrally the septal rudiment is connected with the trabecular commissure only; dorsally it broadens considerably and forms the entire ventral wall of the cranial cavity. The ventral part of the septal rudiment contains those cells (fig. 70 m.b.c.) which at the preceding stage form the medial band dorsally to the trabecular level. These cells now seem to propagate, adding new elements to the septal rudiment. There are no signe indicating that the septum has arisen through lateral compression of the lower part of the membranous cranial wall.

The septal rudiment continues caudad beneath the optic nerves to the hypophysial region (fig. 71), where it gradually becomes lower as the hypophysis nears the epithelium of the mouth cavity towards the hypophysial stalk.

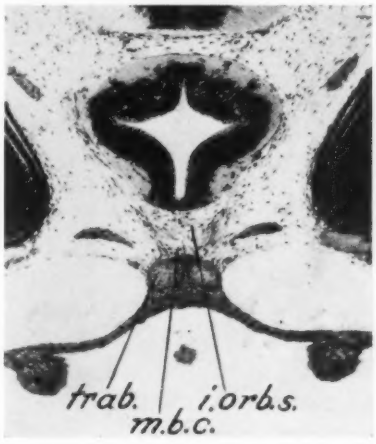


Fig. 70. *Salmo salar*. Embryo, 44 days. Transversal section through the interorbital septum (anterior part).

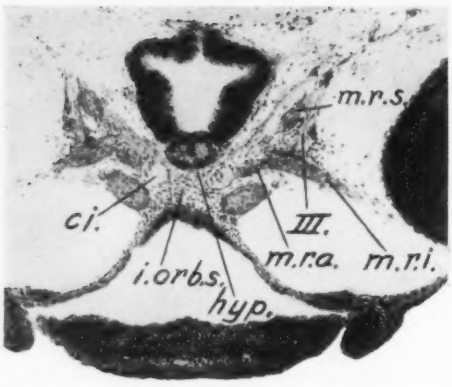


Fig. 71. *Salmo salar.* Embryo, 44 days. Transversal section through the interorbital septum in the region of the hypophysis.

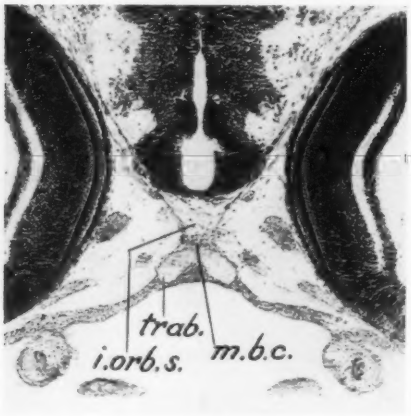


Fig. 72. *Salmo salar.* Embryo, 52 days. Transversal section through the interorbital septum, taken in front of the optic nerve.

On a level with the anterior half of the hypophysis the rectus superior and inferior muscles insert themselves into the membranous wall of the hypophysial sac at the dorsal end of the septal tissue. The rectus internus passes further caudad laterally to this tissue and ends on a level with the posterior border of the hypophysis. The position of the rectus externus is pronouncedly transversal. It ends as at the preceding stage. The lateral commissure is about to chondrify dorsally as well as ventrally. The rectus muscle crosses the commissure at its anterior border.

The eye muscles lie completely externally to those parts of the skull which are clearly defined at this stage.

Stage, 52 days old (newly hatched).

The membranous cranial wall and the septum interorbitale are well defined. The latter extends as at the preceding stage and is rather low. In the territory of the trabecular plate it is attached ventrally to the trabecular commissure portion of this plate (fig. 72). In the region of the paired trabeculae its lower part connects on each side with the medial half of the trabecula or polar cartilage, the lateral half thus forming a pronounced shelf at the lower edge of the septum. The upper part of the septum widens considerably and its lateral angles merge, without a boundary, with the membranous side wall of the brain case. Thus dorsally the septum contains the thickened cranial wall. In the ventral part of the septum it is more densely nucleated than in the rest (m.b.c.). In this part a vivid cell proliferation occurs, at earlier stages originating from the same medial mesenchymatic area as that which furnished the material of the trabecular commissure.

The septum continues caudad, decreasing its height in the hypophysial

region, where its outlines become somewhat obscure as the septum becomes influenced by the rectus superior, inferior and internus muscles of the eye and penetrated by the carotid artery (fig. 73) and the hypophysial vein. Following the upper surface of the septum, i.e. the inner surface of the membranous cranial wall in a fronto-caudal direction, this surface sinks down a little, passing the optic nerve. Further caudally, in the hypophysial region, it sinks down considerably, forming a hypophysial sac. Caudally to the hypophysis the surface rises suddenly straight dorsad, then, turning caudad, forms a sharp angle behind the hypophysis. This angle is the dorsum sellae (fig. 74) or the anterior edge of the still membranous prootic bridge. The prootic bridge is, thus a part of the primary cranial wall. The prootic bridge is quite membranous in this stage of development. Much later it chondrifies from lateral centres[1] lying in the lateral parts of the membrane but well delimited from the prootic region of the cranial cartilage, on to which the centres subsequently fuse.

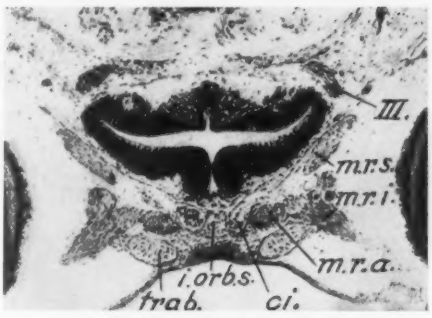


Fig. 73. *Salmo salar*. Embryo, 52 days. Transversal section of the interorbital septum in front of the hypophysis.

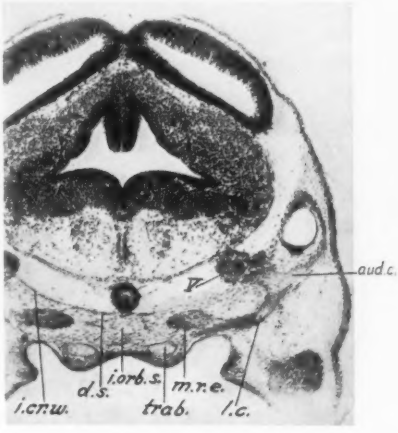


Fig. 74. *Salmo salar*. Embryo, 52 days. Transversal section level with the dorsum sellae and the posterior part of the musculus rectus externus.

The eye muscles behave principally as in the preceding stages, the rectus internus, however, extending further backward along the lateral surface of the septum and ending on a level with the posterior part of the hypophysis. The externus muscle (fig. 74) is located less transversely than at the preceding stages. As at the preceding stages, all the eye muscles are extracranial. A parasphenoid bone is in process of developing in the medial mesenchymatic band. This bone partly covers the trabecular system ventrally. It has not yet reached the hypophysis caudally.

Stage, 58 days old.

The septum and the cranial wall are distinctly outlined. The former is much higher than at the preceding stage (fig. 75). It is considerably thinner vent-

[1] These centres are probably quite independent; at least they are so in *Corydoras* and in *Salmo*.

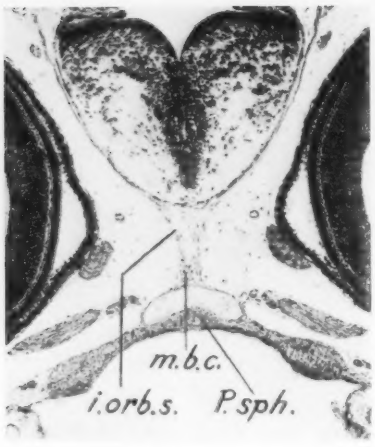


Fig. 75. *Salmo salar*. Embryo, 58 days. Transversal section through the interorbital septum in front of the optic nerve.

rally than dorsally. Its height increases through the activity of the supratrabecular cells of the medial area. The rectus muscles of the eye are inserted as at the preceding stage, but the rectus internus has made an insertion on the inside of the thickened border of the lamina basiotica. Where the membranous lateral wall of the brain-case runs close to the inside of the lateral commissure below the headvein, it adheres to the commissure, so that the rectus externus muscle is delimited from the jugulo-hyomandibular canal (or trigeminofacialis chamber). Thus the externus muscle is enclosed in a canal of its own lying between the lower part of the lateral commissure, (fig. 76) which is extracranial, and the lower part of the membranous side wall of the brain-case. The muscle is thus completely extracranial. The palatinus VII nerve pierces the most lateral part of the membranous prootic bridge (d.s.) and enters the externus canal, i.e. becomes completely extracranial in its further course.

From about the insertion of the rectus inferior muscle at the upper border of the septum (cranial wall) and caudally past the hypophysis, the upper surface of it is fibrous with transversal fibres (fig. 77 h.m.s.). From where the posterior wall of the hypophysial sac turns dorsad to pass into the membranous prootic bridge, the fibrous membrane continues straight caudad as a lamella at the lower side of the posterior septal part (vertical myodom septum). Below the hypophysis the membrane has already broadened, so that its lateral margin has reached the lamina basiotica, to the dorsal surface of which it is attached. This connection is preserved behind the hypophysis, extending to the posterior end of the canal of the externus muscle. The posthypophysial part of the septum forms the vertical myodomic septum along the lateral sides of which the externus muscles extend

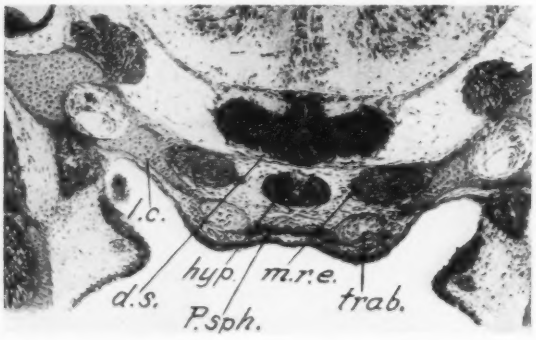


Fig. 76. *Salmo salar*. Embryo, 58 days. Transversal section through the hypophysial regions.

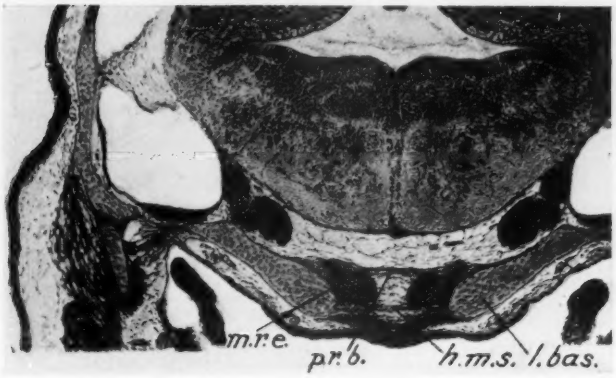


Fig. 77. *Salmo salar*. Embryo, 58 days. Transversal section behind the hypophysial region.

caudad. The fibrous membrane forms the floor of the myodome, the prootic bridge (primary cranial wall) the roof. The myodome is thus completely extracranial even at this stage.

Stage, 23 mm body length.

In all essentials this stage corresponds to the preceding. The m. rectus superior and inferior, however, have altered the position of their insertions. At the preceding stage these muscles were inserted into the upper part of the septum. At the 23 mm stage the rectus superior retains this insertion but has in addition made a strong insertion into the lateral part of the parasphenoid. The rectus inferior has also retained its septal insertion, but a part of this muscle becomes associated with the rectus internus, which it joins and follows nearly to its posterior end below the posterior part of the hypophysis (fig. 78). The rectus inferior is thus found together with the internus in the future ventral compartment of the myodome.

Fig. 78. *Salmo salar*. Embryo, 23 mm. Transversal section through the hypophysial region; with dorsal and ventral compartment of the myodome.

At the 23 mm stage the myodome consists a) of an anterior part, of which the floor consists of the parasphenoid bone, the roof of the ventral part of the septum (horizontal myodomic septum), and the side walls of the polar cartilages, and b) of a

posterior part, of which the floor is the horizontal myodomic septum, the roof the prootic bridge and the side walls the laminae basioticae. Both parts are symmetrically divided by the vertical myodomic septum, which is a backward continuation of the interorbital septum. The anterior part of the myodome contains the posterior parts of m. rectus internus and inferior, the posterior the m. rectus externus. Both parts are entirely extracranial.

Stage, 30 mm body length.

This stage differs from the preceding principally in the further caudal extension of the rectus internus (and inferior) and of the anterior portion of the myodome. The internus muscle has now passed the hypophysis, so that its myodome, behind the hypophysis, lies beneath the myodome compartment of the rectus externus. It is at this stage that the mature condition of the myodome is principally reached. Its posthypophysial part thus consists of the dorsal and ventral compartments separated by the horizontal myodomic membrane, as described by ALLIS and others. At the 30 mm stage the basisphenoid is in process of developing dorsally in the septum and in front of the carotis internus. This bone develops from connective tissue related to the primary insertions of the m. rectus superior and inferior. (In *Esox, Belone, Ammodytes* and others the basisphenoid is cartilaginous, later ossifying.)

From the preceding descriptions the conclusion must be drawn that in Teleosts the myodome is extracranial throughout—a conclusion already expressed by HOLMGREN and STENSIÖ (1936). My investigation has lent no support either to ALLIS' (1919) haemal canal theory or to DE BEER's theories quoted above.

6. *The septum interorbitale and the myodome in Amia and Lepidosteus.*

It has frequently been stated that *Amia* has no septum interorbitale. This statement, however, seems principally to refer to the conditions in relatively old embryos and in adults. In young embryos, 9—11 mm long, there is nevertheless a not inconsiderable septum (not much lower than that in the young *Salmo*-embryo) present (fig. 69) in the anterior part of the orbit. This septum is especially well developed dorsally to the trabecular commissure, it becomes lower beneath the exit of the optic nerve and then disappears further caudad. As in the young embryos of *Salmo* it consists of the thickened ventral wall of the brain-case, which is connected with the medial part of the trabecular plate i.e. with the trabecular commissure. Thus the trabeculae of the orbital region do not take part in the formation of the wall of the brain-case, but form a subocular shelf, exactly as in *Salmo*. This shelf continues throughout the entire orbital region. Where the trabecular plate ends caudally and the paired trabeculae begin, the membranous cranial wall is connected with the

medial border of the trabecula, so that these structures lie almost entirely outside the cranial wall, forming a continous subocular shelf. In the region of the polar cartilage the shelf is especially broad. Caudally this broadened shelf is connected with the anterior end of the lamina basiotica, and with it is connected the lateral commissure and, at later stages, the pila lateralis of DE BEER.

The rectus superior, inferior and internus pass dorsally to the trabecular shelf, being inserted into the membranous cranial wall without forming a myodomic compartment. This condition corresponds to that in young *Salmo* embryos. The rectus externus muscle runs in front of the pila lateralis and the lateral commissure, passing dorsally to the subocular shelf, and enters the deep acute fold found between the shelf and the membranous cranial wall, becoming inserted into the latter. The externus muscle thus lies entirely outside the cranial wall, and the myodome is quite extracranial, just as is the case in *Salmo*.

VOL. 24 1943

There is no septum in a 12 mm *Lepidosteus*. The trabecular plate forms the floor of the cranial cavity, as the membranous lateral wall of the brain-case connects with the plate at its dorsolateral edge. In the region of the paired trabeculae in front of the optic nerves the side walls connect with the entire upper surface of the trabecula and the membranous floor of the brain case forms a connection between the trabeculae. At the optic nerve the side wall connects with the medial half of the dorsal surface of the trabecula, so that the nerve runs over a shelf consisting of the lateral half of the trabecula. This condition is preserved throughout the orbital region. The anterior group of eye-muscles is partly inserted into the basal part of the side wall, partly into the medial half of the polar cartilage. The rectus externus muscle runs dorsally to the shelf formed by the polar cartilage towards the medial half of the polar cartilage and is inserted partly into this cartilage, partly also into the lateral edge of the membranous floor of the brain-case. It lies ventrally to the pituitary vein and its insertion lies somewhat behind the intracranial part of the pituitary vein.

In a 16 mm embryo the conditions are similar, but in front of where the externus muscle runs mediad over the shelf formed by the lateral part of the polar cartilage the membranous side wall of the braincase connects with the entire dorsal surface of the cartilage. Between this shelfless portion and the shelf portion following it immediately caudally a small pocket is formed between the polar cartilage and the side wall of the brain-case. The externus muscle enters this pocket and inserts itself partly at the medial edge of the polar cartilage, partly into the membranous ventral wall of the cranial cavity, which is connected with the medial edge of the polar cartilage. The latter insertion is stronger than the former. The pocket, into which the externus nerve enters, is not deep. It lies in front of the lateral commissure. The

conditions in *Lepidosteus* are much the same as in *Amia,* but the skull floor is broader in the former and the externus muscle therefore does not come as close to the midline as in *Amia.* That is, as far as I can see, the only difference regarding their myodomes.

In older embryos (young fishes) a thick septum has developed in the anterior part of the orbit. This septum owes its development to the action of the anterior and ventral cranial walls, these parts of the wall thickening dorsally to the trabecles. As late as at a 65 mm stage the trabecles of the frontal part of the orbit are still discernible lying close together in the basal part of the septum, the bulk of which is formed through a columnar thickening of the ventral cranial wall. The septum of *Lepidosteus* is much more developed than that of *Amia,* where it is descernible only in young embryos.

7. *Teleosts without a functional myodome.*

Siluroids.

Arius Commersoni and Galidichthys sp. (embryos).

There is no trace of a septum interorbitale, the trabecles forming the ventral wall of the brain-case. The musculus rectus internus (anterior) and inferior are inserted into the thick membrane covering the optic fenestra. The insertion lies below the optic nerve. The rectus externus and superior are attached to the same membrane behind the nerve, the externus immediately outside the trabecular bar, the superior a little higher up. There is thus no trace of a myodome present. In adult specimens of *Pimelodus* and *Amiurus* the conditions are in principle the same as in *Arius* and *Galidichtys,* but in the specimens investigated the brain-case is ossified.

Gadus merlangus, 15 mm.

In *Gadus merlangus* there is a high interorbital septum. Beneath the optic nerve this septum decreases its height considerably, increasing it again somewhat immediately behind the opticus. Towards the hypophysis the septum gradually becomes lower, partially disappearing in front of the hypophysis, where it merges into the hypophysial sac, i.e. the membranous ventral wall of the brain-case. There is no doubt that all the eye muscles insert into the septum extracranially.

A *Gadus morrhua,* a young specimen about 50 mm long, behaves like the 15 mm *Gadus merlangus* and therefore has no myodome.

Anguilla vulgaris, about 100 mm body length.

The common eel has no myodome. Nor is there any interorbital septum present. The rectus muscles of the eye insert in a single group in a very

frontal part of the orbit on a level with the posterior end of the bulbi olfactorii and a long way in front of the entrance of the optic nerves in the brain and a little in front of the point where these nerves pierce the very strong membranous floor of the brain-case. The anterior group of the muscles is inserted between the parasphenoid bone and the floor of the membranous skull, almost meeting medially. The rectus externus is inserted into the most anterior part of the great basisphenoid bone.

Gymnarchus niloticus (embryos)

Gymnarchus has no septum interorbitale. The eye-muscles are collected in an orbital capsule, which also encloses the eye. Outside the cornea the capsule merges into the chorium, which is much thickened in that part. The capsule has two conical expansions, of which the shorter anterior encloses the obliquus muscles, the longer posterior the rectus muscles. The former are inserted into the tip of the anterior cone, the latter in the posterior which extends into a slender tendon, which inserts into the lateral wall of the brain-case below the posterior border of the foramen of the occulomotor nerve. This foramen lies high up on the lateral cranial wall, a considerable distance behind the optic foramen. Consequently there is no myodome present.

Petrocephalus brevipedunculatus and *Marcussenius rudebeckii* behave principally as *Gymnarchus* and have no myodome.

8. *Fishes with only the dorsal compartment of the myodome.*

Corydoras paleatus. In this species is no septum present at any stage of development. The optic fenestra is covered with membrane. From a point on a level with the optic nerve this membrane connects not with the lateral border of the trabecular bar but with its upper surface, thus giving it the appearance of a subocular shelf. Caudally this shelf is broader and merges into the lateral commissure. The anterior group of the eye muscles is inserted into the trabecular bar in the acute fold between the shelf and the membranous side wall of the brain-case. The m. rectus externus has (in a 6 mm embryo) a similar insertion, but as the shelf is very broad at the point of insertion and the muscle has a mediocaudal direction, it has the appearance of being intracranial; as, however, it lies completely external to the brain-case, it must be extracranial. The conditions regarding the externus muscle are much the same as in *Amia.* At later embryonic stages, when the parasphenoid has developed a lamelliform ridge which covers the inside of the ventromedial part of the trabecular bar, the externus muscle shifts to this ridge.

Gadus merlangus, about 30 mm.

In this embryo the external rectus muscle was found to continue caudad between the ventral wall of the brain-case and the parasphenoid bone, ending

VOL. 24 1943

beneath the hypophysis almost on a level with its posterior surface. As the membranous wall of the skull has connected with the medial surface of the lamina basiotica (or the polar cartilage?) along a line almost parallel with the muscle, this muscle has become enclosed in a subcranial canal, the floor of which is the parasphenoid bone. Thus a myodome is present corresponding to the dorsal compartment in *Salmo*. The other rectus muscles insert into the septum well in front of the hypophysial sac.

In *Glaridichthys reticulatus* the conditions are much the same as in *Amia*. The anterior group of rectus muscules is inserted into the lateral portion of the outside of the parasphenoid bone, which has become incorporated in the wall of the brain-case. The rectus externus enters an extracranial myodomic canal, as in *Amia*.

In the broad division of the trabecular plate there is no trace of a septum interorbitale, the membranous lateral wall of the brain-case joining directly on to the dorso-lateral edge of the plate. In the narrow division of the united trabecula a very low and broad septum appears dorsally to the trabecles. This septum gradually becomes somewhat higher towards the optic foramen. Its tissue simultaneously becomes denser, tendinous. Almost immediately in front of the opticus the septum carries on each side a long, very thick and strong ligamentum tenaculum oculi, which extends to the eye bulb, where it spreads out desklike to form a part of the sclerotic membrane of the eye. Immediately behind the ligamentum the septum is represented by very spaciously fibrillated and nucleated tissue continous with the membranous cranial wall. This tissue seems to disappear as a distinct component already in front of the level of the anterior part of the parasphenoid wings. These observations have been made principally on a 13.5 mm embryo, but the conditions are practically the same in embryos of 5, 6, 7, 7.5, 8.5, 9.5 and 10 mm body length.

9. *Fishes with a complete teleostean myodome.*

The bulk of teleosts have a myodome which more or less agrees with that in *Salmo*, and therefore will not be treated here specially.

10. *Conclusions.*

In the development of the myodome in *Salmo salar* three stages may be recognized: (1) without a myodome (*Polypterus*-typus), (2) with a myodome containing the m. rectus externus only (*Amia*-typus) and (3) with a myodome containing all the rectus muscles lodged in a dorsal and a ventral compartment (normal teleostean typus).

Each of these three ontogenetical stages corresponds to a definitive myodome condition. The first stage answers to the conditions in Selachians,

Crossopterygians, most Siluroids, *Anguilla, Gadus morrhua,* and perhaps other teleosts. The second stage is represented by Palaeoniscids (probably), *Amia,* the siluroid *Corydoras, Gadus merlangus, Glaridichthys* and probably many others. The third stage is the general teleostean stage.

The list of teleosts treated here, incomplete as it is, nevertheless contains some noteworthy facts, which will now be considered at some length. *Gadus merlangus* and *morrhua* represent the second and first stages respectively. This remarkable difference between two species of the same genus seems to indicate that there is no strict coincidence between ontogeny and phylogeny regarding the myodome conditions. The same must be the case with the cypriniform teleosts, in which the Cyprinids and Carassidids have a complete teleostean myodome, whereas the Silurids either have no myodome at all or a myodome of the second type. Nevertheless, as the cypriniform fishes have Weberian ossicles they must form a natural unit. Concerning the Silurids (*Amiurus*), ALLIS (1909) believed that they behave primarily since they have no myodome, as is also the case in *Lepidosteus* and *Polypterus.* If, however, the absence of the myodome in the Silurids is primary, either the Weberian bones or the myodome must have developed independently in Cyprinids and Silurids (ALLIS, 1909). DE BEER (1926) seems to have solved this dilemma by assuming that the silurid condition is due to a reduction of a pre-existing myodome. This theoretical conclusion, however, is not supported by any ontogenetical evidence, as in the development of such fishes devoid of a myodome as Silurids (and *Gymnarchus?*) there are no traces of a pre-existing myodome present. But this is not a good argument against DE BEER's theory, as such traces need not necessary have been left even if the species in question sprang from an ancestry possessing a complete myodome. A certain complication of DE BEER's theory signifies the fact that an interorbital septum is absent and the skull is platybasic in Silurids and *Gymnarchus,* as is the case in *Polypterus,* in which probably a myodome is primarily absent. In the other cypriniform fishes a high interorbital septum is present and the skull is tropibasic. In teleosts and ganoid fishes the development of the skull generally begins with a platybasic stage, which, however, soon develops into a tropibasic. In *Leuciscus rutilus* the skull of a 5 mm embryo is already tropibasic (HUBENDICK, 1942). At this stage, which I have had an opportunity of investigating, the interorbital septum is very insignificant, consisting of the thickened medio-ventral wall of the membranous cranial wall joining the medial edge of the trabeculae and without any ventral part belonging to the medial area of the head. Thus the septum is, practically speaking, absent. (The 5 mm stage is the earliest stage of *Leuciscus* that I have examined.) As the development of the myodome is throughout the same as in *Salmo,* it may be permitted to assume that at a stage earlier than that of 5 mm the membranous skull had not yet formed as in early platybasic stages of *Salmo,*

VOL. 24 1943

where the brain is surrounded by the general mesenchyme of the head, which is also connected with the rudiments of the trabeculae. As the brain-case is formed through the condensation of a part of the mesenchyme, the medial part of the trabeculae is incorporated in the wall of the case in *Salmo* and *Leuciscus,* whereas in the platybasic skull of *Corydoras* the entire trabecula is incorporated. Thus the difference is but gradual. Thus, *Corydoras* (and the other silurids) has, as far as the basis cranii is concerned, checked its development at a stage corresponding to the first stage of the ontogenetical development (no myodome) of the general teleostean type.

In *Gadus* two types of myodomic development are present. In both cases there is a high interorbital septum, but in *G. merlangus* (30 mm) a myodome (containing the rectus externus muscle) is present, in *morrhua* there is none. In that case it seems to be a question of a reduction of the myodome in the latter, such conditions being retained in the adult, which are characteristic of the 15 mm stage of *merlangus* (1st stage of ontogeny, no myodome). In *Glaridichthys* (*Cyprinodontidae*) a very low interorbital septum is present and the myodome belongs to the second ontogenetical type. This fish thus has the same myodonic condition as *Gadus merlangus* and its myodome may have developed from a general teleostean type.

Now the question arises: How are we to explain the exceptions from the general teleostean type recorded in the preceding lines? The conditions in Cyprinids and Silurids give the clearest answer to this question. On the one hand these groups have Weberian ossicles and are therefore closely related, on the other hand the Cyprinids have a complete myodome, the Silurids no such structure. As the Weberian ossicles cannot have originated independently in the two groups, they must have sprung from an ancestry with those ossicles. As the Cyprinids have a complete myodome of a teleostean type, they must have developed from Teleosts with a complete myodome, and thus the Silurids must also have developed from ancestors with a complete myodome. In the ontogeny of the siluroid fishes there is not the slightest indication of their having once had a myodome. Nevertheless they must be derived from fishes with a myodome. How then can we explain this contradiction? I cannot find any explanation other than that the Siluroids have developed from the cyprinid stem, checking their development in regard to the myodome already at the ontogenetically first stage of its general teleostean development. The other exceptions from the general teleostean myodome type may be simply explained in the same way. None of them has ever had a fully developed myodome in spite of their evolution from fishes with a complete myodome.

It remains now to consider the Ganoids and *Polypterus.*

The myodome of *Amia* belongs to the second ontogenetical stage and has passed the first stage of its development. There is no septum interorbitale in

VOL
24
194

the adult fish, but a rudimentary septum is present in young ontogenetical stages. The trabecula of the embryos and young fishes (at least) are not fully incorporated in the cranial wall but their lateral parts form a fairly strongly developed subocular shelf. The presence of this shelf may perhaps be regarded as a token of a preexisting septum interorbitale, but it may also be considered to be a step towards a developing septum. I prefer the first interpretation because of the existence of a septum in *Lepidosteus* and in palaeoniscids fishes such as *Saurichthys, Perleidus* and *Ospia* (STENSIÖ, 1925, 1932). As the palaeoniscids are probably forerunners of the Ganoids, the existence of an interorbital septum in the latter may be regarded as a primary character when it comes into existence in the embryo of *Amia* or in the adult of *Lepidosteus.*

The very large myodome of *Perleidus* has, like the Kansas Palaeoniscid A of WATSON, two entrances according to STENSIÖ, viz. an anterior and a lateral. The two openings are separated by a skeletal rod called by STENSIÖ the lateral paired rod of the pars basisphenoidea. The lateral opening is called the main, the other the medial aperture. These conditions very strongly recall those in *Amia* 41 mm illustrated by DE BEER (1926), but there the medial basisphenoid rod is absent or rudimentary and the lateral rod is represented by the pila lateralis (processus ascendens palato-quadrati). The rectus externus muscle of the eye enters the myodome medially to the pila. The lateral opening of the myodome is the entrance to the trigemina-facialis chambre, inside the lateral commissure. The conditions in *Perleidus* are so similar that I cannot doubt but that its interpretation must be the same. Thus the lateral rod of the basisphenoid is probably the pila lateralis (processus ascendens, alisphenoid pedicle of ALLIS). In *Ospia* and *Saurichthys,* in which the lateral rod is missing, the myodome has only one large aperture. The r. ophthalmicus profundus V of *Ospia* has its exit through a canal of its own in the posterior part of the orbit. From the intracranial course of this nerve STENSIÖ (1932) concludes that the entire trigeminal ganglionic complex had an intracranial position. This conclusion does not appear to be correct, as the profundus nerve in *Amia* as well as in *Polypterus* issues directly from the brain and to begin with runs intracranially, in spite of the extracranial position of the trigeminal complex (inside the lateral commissure in *Amia*). I believe therefore that the posterior lateral part of the myodome contained at least part of the trigeminal ganglionic complex, as it does in *Amia.*

It is a well-known fact that in *Polypterus* there is no trace of a myodome. As the development of the skull of this fish is but incompletely known, one cannot tell wether its development is subject to conditions which can be interpreted as rudiments of a pre-existing myodome. The most probable assumption, however, is that *Polypterus,* like the Crossopterygians, is primarily devoid of a myodome, as was assumed for instance by ALLIS.

NILS HOLMGREN

CONCERNING THE ANTERIOR BOUNDARY OF THE ORBIT IN SELACHIANS AND TELEOSTOMIANS.

In the first part of this work (HOLMGREN, 1940) I have followed the development of the lamina orbito-nasalis and of the anterior sideplate in sharks and rays. The orbitio-nasal lamina always has an independent rudiment. In squaloid sharks it consists of three parts, a posterior, a medium and an anterior. The posterior part forms the ectethmoid process, the medium the anteroventral (anterior) part of the frame of the orbit and the anterior the nasal capsule. In *Scyllium* the posterior part is rudimentary and the medium and anterior parts form one unity, the thickened posterior border of which becomes the anterior orbital frame and the anterior portion the nasal capsule. In *Raja* and *Urolophus* the orbito-nasal lamina forms the nasal capsule. In *Torpedo* a bandlike part of the lamina forms the anterior frame of the orbit, an anterior expansion of which becomes the nasal capsule. In all rays there is no ectethmoid process.

Behind and internally to the orbito-nasal lamina lies the anterior side-plate. It is formed by a band of tissue connecting the trabecula with the rudiment of the orbital cartilage. It is always separated from the lamina orbito-nasalis (anterior frame of the orbit) by the orbitonasal canal. In sharks the anterior cerebral vein generally lies posteriorly to the side-plate and the m. obliquus is inserted inferiorly into it. In *Heterodontus,* however, the anterior cerebral vein foramen seems to lie in front of the side plate, as in *Raja* and *Torpedo.* The anterior side plate belongs to the wall of the brain-case, and the olfactory bulb and nerve thus lie internally to the plate. In *Raja* and *Torpedo* the anterior cerebral vein lies anteriorly to the side plate, in *Urolopus* the plate has a portion anteriorly and another posteriorly to the vein foramen. *Urolopus* thus has an intermediate position between the other rays and the sharks as far as regards the position of the vein. In the rays the orbito-nasal canal always runs between the lamina orbito-nasalis and the side-plate, as in sharks.

In different teleostomian fishes HAMMARBERG (1937) has described two structures at the anterior boundary of the orbit, viz. the ectethmoid and the entethmoid processes. The ectethmoid process is the orbito-nasal lamina of DE BEER (1927), but not the parethmoidal process of PEHRSON (1922), as DE BEER maintains. The parethmoidal process of PEHRSON is at a 10—11 mm stage of *Amia* a process "directed obliquely upwards and outwards with its inner side lying close to the brain". The lamina orbito-nasalis of DE BEER is also present in Amia. PEHRSON describes it in the following way: "from the margin of the orbito-nasal plate and at right angles to it a pair of longitudinal cartilaginous processes form, which merge into the parethmoidal ridges. A short canal, open at both ends, is hereby formed between the processes, the

ridges and the dorsal side of the orbito-nasal plate. The Mm obliqui inferiores and superiores find their basal attachement in the canals." PEHRSON gives no name to the process which forms the external wall of this canal, but it cannot be doubted that it is the ectethmoid process or the lamina orbito-nasalis of DE BEER.

The argument upon which DE BEER has based his opinion of the ectethmoid process corresponding to the lamina orbito-nasalis in Selachians, is that in both cases it is formed as a process emanating from the trabecula. In neither case does this hold true. In my paper of 1940 I have shown that the lamina orbito-nasalis develops from a separate blastema fusing on to the trabecula and the anterior end of the orbital cartilage. In the teleostomians I have also found the ectethmoid process developing from a separate blastema immediately posteriorly to the olfactory sac. This blastema chondrifies later in connection with the ethmoidal plate and has probably therefore been described as a process of this plate. The basis of DE BEER's homologization is therefore incorrect, but the conclusion is nevertheless correct.

The parethmoidal horns of PEHRSON (cornua parethmoidalia) and the "entethmoid process" of HAMMARBERG are, according to the latter author, synonymous. I am of the same opinion.

From the preceding lines it seems possible to draw the conclusion that the orbito-nasal lamina of selachians is homologous with the ectethmoid process in teleostomians, and the anterior side plate of selachians with the "entethmoid" process or parethmoidal process in teleostomians. Thus the canal separating the two structures in teleostomians must probably be regarded as homologous. Additional evidence for this conclusion is offered by the fact that an orbito-nasal vein, falling into the lower orbital sinus, and the orbito-nasal artery (of the external carotid system) run through the canal in Selachians as well as in Teleostomians. (In *Salmo* a dorsal vein also is present running to the dorsal orbital sinus.)

The lamina orbito-nasalis of Teleostomians seems to correspond closely with the posterior border portion of that of *Scyllium*.

THE ORBITAL CAPSULE AND ITS RELATIONS TO THE SKULL.

1. *In Petromyzon and in Selachians.*

The orbital capsule of *Petromyzon* is well developed. It has been described by DÜCKER (1924) as a sphaerical connective tissue capsule, in the formation of which the cartilage of the skull takes but an inconsiderable part. A shallow concavity of the skull and a part of the "Nasenkapselknorpel" give some support to the eye bulb. The capsule consists of coarse, principally meridionally arranged fibres.

The descriptions of TRETJAKOFF (1926) and of FRANZ (1932) give no essential additional material to the question of the morphology of the orbital capsule in *Petromyzon*. From FRANZ' paper I gather that according to him the orbital capsule should be present only in *Petromyzon:* "Die bindegewebige Orbitalkapsel, diese Eigentümlichkeit von *Petromyzon*, . . ."

In a very young *Ammocoetes* (-larva) the eye together with the eyemuscles is surrounded by a strong connective tissue membrane, issuing from the skin. The dorsal wall of this membrane is attached on to the membranous brain-case dorsally, but before attaching it sends down a lamella outside the ophthalmic nerve parallel with the lateral wall of the brain-case. At the central part of the orbit the lamella seems to be fused with the cranial wall. The medial wall of the orbital capsule ends ventrally in a connective tissue mass which is connected with the trabecula. From this part issues the ventral wall of the capsule. This wall is very thick and attaches to the skin ventrally to the eye.

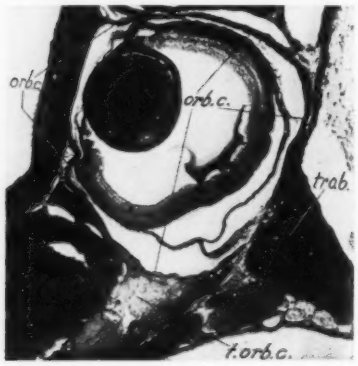


Fig. 79. *Petromyzon planeri*. Ammocoetus-larva. Transversal section through the orbit.

In an old *Ammocoetes*, near metamorphosis, the orbital capsule is essentially as in the young larva, but has widened considerably as the eye has grown larger. the dorsal wall has thinned considerably but else behaves as in the young larva in relation to the ophthalmic nerve and the cranial wall. The ventral wall is also thinner than before with exception of a portion ventrally to the eye bulb, where it is very thick (fig. 79 t.orb.c.). This thick portion frontally joins on to the trabecular mesenchyme mass and ends caudally in the mesenchymatic mass dorsally to the root of the trigeminus (in front of the pedicle process). This thickened part later will be substituted by the "subocular arch". The trigeminal nerve never enters the capsule, there always being a portion of the capsule wall separating from the space in which the eye and eye-muscles are situated. The suborbital part of the trigeminal (trigeminal II) is surrounded by a thick membrane of which the dorsal wall is the capsular wall and the ventral one a connective tissue membrane connected with the capsule on both sides of the nerve. The orbital capsule encloses wide upper and lower venous sinuses.

In the adult *Petromyzon* the conditions are as in the larva, but the thickened part of the ventral wall of the orbital capsule now encloses a cartilage (fig. 80 s.o.a.), viz. the "subocular arch". The ophthalmic nerve seems to have entered the capsule, as the thin lateral wall of the canal in which it was enclosed seems to have disappeared partly, especially in the posterior part of

the orbit. The trigeminal II always runs outside the capsule in a canal of its own, as in the larva.

Comparing the orbital capsule of the *Petromyzon* larva with the orbital capsule sac in *Anguilla* (fig. 83) the only difference seems to be that the ventral wall in *Petromyzon* has a longitudinal thickened part, whereas *Anguilla* has no such thickening. (See p. 177.) In the teleosts the anterior wall of the orbital capsule ends at the anterior border of the anterior myodome, i.e. at the orbitonasal lamina. In the adult *Petromyzon* the capsule is frontally connected with the anterior pillar of the subocular arch. Then the question arises: Is it possible that the anterior pillar of the subocular arch represents the orbital lamina (ectethmoid) in teleosts? In such a case a ventral part of the orbital capsule should perhaps represent the subocular arch (epitrematic bar). In order to get an answer to this question, we may now consider the conditions in a 48 mm embryo of *Squalus* and in a young *Heptanchus* (75 mm). In my paper of 1940 I have shown that the orbitonasal lamina and the membranous antorbital process are in Selachians formed from the mesenchyme of the lower eye-brow area. The membrane of the antorbital process in *Squalus* (48 mm) is frontally joined with the orbitonasal lamina. Caudally it extends below the eye, a good distance below the limiting membrane (orbital capsule) of the orbit. The antorbital process, seen in transversal section, consists of four parts (lamellae) radiating from a centre. One of these lamellae joins with the orbito-nasal lamina (ectethmoid process) and is thus directed mediad and caudad; another, dorsal and shorter, joins with the orbital capsule below the middle of the orbit, the third extending laterad to the ventral limb of the supraorbital canal and then to the lower eye brow and the fourth being directed towards the infraorbital canal and joining with its mesenchymatic envelope. Where the four limbs meet the mesenchyme is thicker than in the lamellae proper, forming a central axis of the antorbital process. In *Heptanchus* the antorbital process is chondrified and frontally fused on to the lamina orbito-nasalis, as I have stated in my paper of 1941. In the 75 mm embryo the posterior attenuated part of the antorbital cartilage lies in the wall of the orbital capsule (fig. 81), whereas the anterior part is removed a little from it (fig. 82). A transversal section through the anterior part of the orbit thus agrees perfectly with a corresponding section in *Petromyzon*,

Fig. 80. *Petromyzon fluviatilis*. Adult. Orbital capsule. Transversal section.

especially as the other parts of the orbital capsule are formed correspondingly in the two genera.

In *Heptanchus* the antorbital cartilage (capsular part) is joined with the infraorbital canal envelope by means of a thick lamella of not very densely crowded mesenchymatic cells. From this lamella issues a thin but very distinct lamella, which runs out into the lower eye brow. A strong medially directed lamella also issues from the distal part of the thick lamella mentioned above.

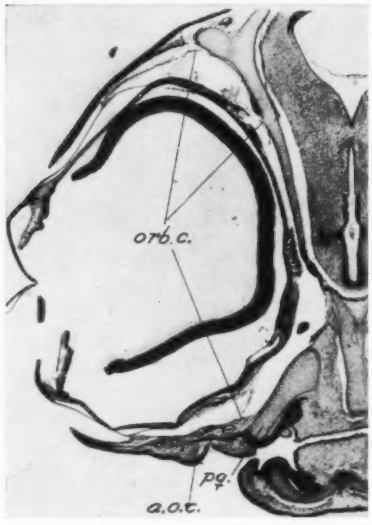


Fig. 81. *Heptanchus cinereus.* Embryo 75 mm. Transversal section through the orbit.

Fig. 82. *Heptanchus cinereus.* Embryo 75 mm. Transversal section through the orbit, in front of that in fig. 81.

This medial lamella inserts into the tendinous band which joins the labial cartilage with the palato-quadrate. Comparing with the radiating lamellae in *Squalus,* it seems obvious that the conditions in *Heptanchus* are principally the same, the four (radiating) lamellae of *Squalus* being present in *Heptanchus* even if somewhat differently arranged. The connection with the labial cartilages is, however, absent in *Squalus.*

The differences between *Squalus* and *Heptanchus* may be interpreted in the following way. In *Squalus* the part of the cheek which lies between the eye and the mouth is much higher than in *Heptanchus.* Therefore is the complexity of lamellae described in the preceding lines much more compressed in *Heptanchus* than in *Squalus.* The antorbital cartilage in *Heptanchus* lies in the orbital capsule membrane, the antorbital rudiment (membrane) distanced from this capsule in *Squalus.* The difference may be explained by the difference in the hight of the cheek in the two genera, the rudiment in *Squalus* having space for its separation from the capsule and for its shifting

to the centre of the cheek mesenchyme, preserving only a weak lamellar connection with the orbital capsule.

Comparing the conditions in *Heptanchus* with those in *Petromyzon,* one is struck by the great agreements. The capsule proper has a corresponding extension. In the ventral wall a cartilage is present in both cases and the anterior wall of the capsule is connected with the anterior part of this cartilage or its frontomesial continuation. In *Petromyzon* this cartilage forms the subocular arch, in *Heptanchus* the antorbital cartilage + the lamina orbitonasalis. In *Petromyzon* the anterior wall of the orbital capsule connects with the anterior pillar of the subocular arch, in *Heptanchus* with the posterior border of the orbitonasal lamina. The conclusion wich may be drawn from these agreements must be that the epitrematic bar (longitudinal part of the subocular arch) is represented in *Heptanchus* by the antorbital cartilage and the anterior pillar of the subocular arch by the lamina orbitonasalis. There is, however, the difference that in *Heptanchus* the lamina orbitonasalis carries the nasal capsule, whereas this capsule in *Petromyzon* is independent of the anterior pillar of the subocular arch. But if the olfactory organ is secondarily unpaired in *Petromyzon* (SEWERTZOFF and TRETJAKOFF) and the nasal capsule thus also is secondarily unpaired, it may be possible that its independence from the lamina orbitonasalis also is secondary. In my paper of 1940 I have pointed out that in Selachians there is an independent rudimentary primary olfactory capsule present at stages where the definitive olfactory capsule is not jet discernible. It is then very possible that the capsule in *Petromyzon* is the two primary capsules fused into one. If the nasal capsule in *Petromyzon* is such a primary capsule, it is easily understood that it has no connection with the orbito-nasal lamina, the rudimentary primary capsule in Selachians having no relation to their orbito-nasal lamina.

The preceding conclusions—the homology between orbito-nasal lamina + antorbital cartilage and the subocular arch—bring us consequently to the conclusion that the antorbital cartilage + the orbitonasal lamina are extravisceral structures comparable with the extrabranchials in *Petromyzon* and *Myxine,* the antorbital cartilage in *Heptanchus* being the epitrematic bar and the orbito-nasal lamina the socalled 3d prebranchial arch of SEWERTZOFF.

In my paper of 1942 I have made an attempt to compare the labial cartilages in the *Holocephali* with the buccal skeleton of *Petromyzon.* Assuming that SEWERTZOFF's opinion regarding the labial cartilages in sharks was correct, viz. that the posterior upper labial cartilage corresponds to the 3d prebranchial arch (anterior pillar of the subocular arch) and the anterior upper to the posterior lateral cartilage, I tried to deduct the homologies of the other "labial cartilages" in the *Holocephali* from the complicated buccal skeleton in *Petromyzon.* The result of the preceding discussion calls for a revision of

VOL. 24 1943

my homologisation (HOLMGREN, 1942), as the posterior labial cartilage cannot be homologous with the 3d prebranchial arch or the lamina orbito-nasalis, as both are present simultanously in sharks. Then the question of the homologies of the labial cartilages in sharks arises. In my paper of 1942 I have assumed that there was originally two upper labial cartilages present in elasmobranch fishes and on this basis I have attempted to interprete the single upper labial cartilage of *Notorhynchus* and *Narke* as well as of Chimaeroids as consisting of the two upper labials of sharks fused together. As I have, however, remarked that as long as the ontogenetical development of the upper labial of *Notorhynchus* is not known, the conclusion of it as composed of two fused cartilages "is somewhat uncertain". The same applies to the upper labial of *Narke* and Chimaeroids. In *Heptanchus* but one small upper labial cartilage is present. Examining my 75 mm embryo I have found a small cartilage nodule lying immediately in front of the relatively well developed rudiment of the labial cartilage. This nodule is not present in the adult and therefore must have fused on to the larger cartilage. This finding seems to confirm my earlier interpretation of the upper labial cartilage in *Notorhynchus* as being composed by two upper cartilages. The same may be the case in *Narke* and the *Holocephali.*

If the "subocular arch" of *Petromyzon* represents the lamina orbito-nasalis + the antorbital cartilage of *Heptanchus,* it is immediately clear that the 3d prebranchial arch (anterior pillar of the subocular arch) cannot be the homologue of the posterior labial cartilage in sharks. Thus the supposition which served as basis for my earlier attempt (HOLMGREN, 1942) to homologize the pieces of the buccal skeleton of *Petromyzon* with those of the labial skeleton in Holocephalians, cannot be correct. I must therefore look for other possibilities. In my paper of 1940 I have followed the development of the labial cartilages in sharks. They were found developing in near relation to the early connection between the trabecula and the palato-quadrate blastema, thus also to a frontal part of the trabecula. It therefore seems most likely that the labials, if present in *Petromyzon,* should have some relation to the trabecula. As the buccal cartilages lie in front of the anterior end of the trabeculae, it seems excluded that any of them might represent labial cartilages. I am therefore now inclined to assume that *Petromyzon* was devoid of labial cartilages. On the other hand *Myxine* possibly has a representative of a labial cartilage, as the so-called *cornual cartilage* bears a certain relation to the trabecula which might possibly correspond to that of a labial cartilage.

Assuming that the labial cartilages of sharks and Holocephalians were unrepresented in *Petromyzon,* there remain in the Holocephalians and in *Petromyzon* four cartilages which perhaps are comparable in the following way:

Petromyzon	*Holocephalian*
Posterior lateral cartilage	pedicle cartilage
Posterior dorsal plate	prelabial cartilage
Anterior dorsal plate	prenasal cartilage
Annular cartilage	premaxillary cartilage

The anterior lateral cartilage of *Petromyzon*, which belongs to a deep skeletal layer developed close to the lining epithelium of the buccal cavity, probably does not belong to the buccal skeleton.

It is not necessary to emphasize that the preceding comparison is only a very objectionable attempt to a working hypothesis, as was also the "daring comparison" of my earlier work (HOLMGREN, 1942).

2. *In Teleostomians.*

Anguilla vulgaris.

The orbital capsule is strongly developed (fig. 83). Its dorsal wall runs from the upper border of the cornea past the bone-cylinder of the supraorbital sensory line, to the upper border of the membranous orbital wall. There is also a medial connection with the lateral border of the frontal bone below the bone-cylinder. Before its insertion on the frontal bone it sends off a lamella in a ventro-medial direction externally to the ophthalmicus superficialis VII (o.p.s.) nerve and the supraorbital vein. In this way a canal is formed for the nerve and the vein. This lamella is extremely thin or partly missing in the medium part of the orbit. Frontally it is thicker. The ventral border of the lamella connects with the membranous orbital wall and simultanously becomes thicker. It then covers the outside of the cranial wall as a thick collagenous layer, which partly might be fused on to this wall. Ventrally the cranial wall and the medial wall of the orbital capsule separate. The ventral border of the capsule joins a short horizontal membrane (h.mb.) or collagenous mass, which joins the unpaired trabecula, and behind this the membrane nears the midline or the lateral border of the anterior end of the basisphenoid bone. The ventral wall is thick but not as thick as the dorsal. It begins laterally at the ventral border of the cornea, runs mediad, ventrally to the ventral orbital sinus, and joins the medial wall as already mentioned. The anterior end of the orbital capsule joins the anterior upper and

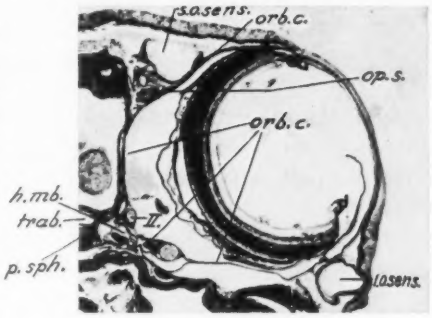


Fig. 83. *Anguilla vulgaris*. Young fish. Transversal section through the orbital capsule.

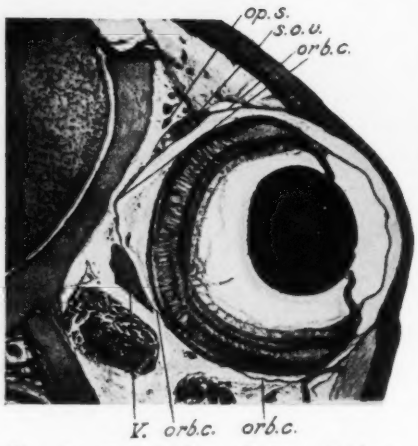


Fig. 84. *Gymnarchus niloticus.* Embryo. Transversal section through the orbital capsule.

lower borders of the anterior myodome. The posterior part of the sac laterally joins the upper part of the postorbital sensory line bone-cylinder and medially the anterior border of the membranous lateral commissure. The trigeminal nerve runs entirely outside the orbital capsule. Its maxillo-mandibular branch is enclosed in a membranous canal immediately below the orbital capsule, as is the case in *Petromyzon*. A comparison with the latter results in the statement that there is a good agreement betveen *Anguilla* and *Petromyzon* but with the exception that there is no cartilaginous part enclosed in the ventral wall of the capsule in the former. In *Petromyzon* the capsule joins the 2d prebranchial arch ("mandibular arch") whereas it joins the anterior edge of the membranous lateral commissure (processus oticus internus of the mandibular arch) in *Anguilla*.

Gymnarchus niloticus.

As already pointed out (p. 165) the eye muscles of *Gymnarchus* embryos (fig. 84) are enclosed in an orbital capsule. This capsule is more or less conical in that part which encloses the rectus muscles, but has a frontal likewise conical prolongation enclosing the obliquus muscles. The rectus muscles have their origin at the conical end of the capsule, which is attached by means of a slender tendon to the neurocranium immediately below the foramen for the oculomotorius nerve. The obliquus group is attached dorsally to the posterior border of the olfactory foramen. The capsule is formed by a comparatively thick membrane and is filled up by a sparce web of mesenchymatic cells. Excepting the eye muscles the capsule also contains their nerves and the optic nerve. Wide and rather well delimited spaces in the periferal part of the capsule dorsally as well as ventrally to the optic nerve contain blood-corpuscles in a young (41 mm) embryo. These spaces therefore represent a dorsal and a ventral orbital sinus. The capsule is connected with the skull only at those points mentioned above.

In a 58 mm embryo the orbital capsule is in contact, but without fusion, with the orbital wall on a territory in front of the foramen of the anterior cerebral vein and on a level with the posterior part of the eye.

Laterally the orbital capsule embraces the eye-bulb and joins the skin around the border of the cornea. The skin consists of the thick epidermal

layers, a thick chorium and a thick subdermal connective tissue layer which is bordered internally by a strong collagenous membrane at the 58 mm stage. At the 58 mm stage the capsule is connected with this membrane dorsally as well as ventrally to the eye. The membrane connects with the supraorbital sensory line bone (frontal bone) and the upper border of the endocranium and ventrally to the eye with the infraorbital sensory line bone, which lies close to the orbital capsule. There is thus dorsally to the eye formed a canal, triangular in transverse section, formed by the capsule, the collagenous membrane and the neural skull, which encloses the ophthalmicus superficialis nerve and the supraorbital vein. This canal is very wide caudally but narrows considerably where the capsule is in contact with the orbital wall.

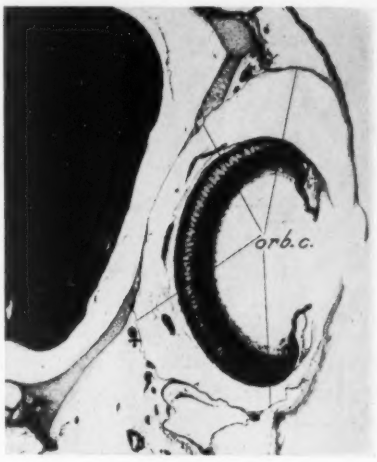


Fig. 85. *Petrocephalus brevipedunculatus.* Young fish. Transversal section through the orbital capsule.

VOL. 24 1943

Petrocephalus brevipedunculatus.

Petrocephalus (fig. 85) behaves principally as *Gymnarchus* regarding the orbital capsule, but with some differences. The sac is much wider than in *Gymnarchus* probably owing to the greater width of the orbital sinuses which expand the sac especially medially, where it is forced nearer to the neurocranial wall, so that the territory of contact is much larger than in *Gymnarchus*. That collagenous membrane which connects the capsule with the supraorbital sensory line bone in *Gymnarchus* is much shorter so that the triangular canal at the upper border of the orbit is much narrower in *Petrocephalus* than in *Gymnarchus*. In the territory of contact the capsule is very thin, but the capsular membrane can mostly be followed separated from the membranous neurocranial wall. Only in the centre of the territory the both membranes are completely fused on to one another. The ventral wall of the capsule is very thin. It is laterally in close contact with a broad lamella of the infraorbital sensory line bone or is perhaps fused with the thin periost of this bone. Medially the membrane extends to the cranial wall on a level with the optic foramen. The anterior wall of the capsule is thickened and extends frontad to where the obliquus muscles of the eye have their origin, a short distance behind the nasal sac. The posterior wall of the eye-muscle sac is also rather strong. Its most posterior lateral part is connected through a short membrane with the postorbital sensory line bone.

Teleostomians, generally.

In the teleosts the ventral wall of the orbital capsule is either extremely thin or reduced to a short lateral membrane which connects with the lateral border of the lower orbital sinus, the endothelian cells of the sinus substituting the missing capsular wall. The ophthalmic canal is devoid of a lateral wall in most teleosts.

LIGAMENTUM TENACULUM OCULI.

The "Ligamentum tenaculum oculi" in teleosts has been described by early authors as a ligament joining the skull with the bulbus oculi. It has been described later by HARMAN (1899), who found it present in all fresh water fishes and in a great number of other fishes. The "ligamentum tenaculum oculi" is not homologous with the eye-stalk of Selachians, as it is present together with the eye stalk in *Raja* [quoted from FRANZ (1934)].

In my material of *Salmo salar* I was able to follow the development of the "ligamentum". In a 15.5 mm *Salmo* it begins to appear. At this stage the optic nerve runs out from the brain case in a lateral direction straight towards the eye bulb, but before entering it the nerve makes a short bend ventrad. The optic nerve is surrounded by a thin layer of mesenchymatic cells, which is thicker on the frontal surface of the nerve and there sends off a number of fibres directly to the sclera of the eye. At a 16 mm stage these latter fibres form a rather strong bundle, which at the 16.5—19 mm stages gradually separates from the connective tissue tube surrounding the optic nerve. At later stages the ligament becomes even more separated from the opticus and forms an immediate connection between the upper end of the septum interorbitale immediately in front of the exit of the optic nerve.

In Selachians no ligamentum tenaculum oculi is present either in embryonic sharks or in rays. In a *Raja clavata* of 90 mm body length no differentiated ligament is present. But in this specimen it is clearly seen that the anterior wall of the opticus tube is much thicker than the other parts of the tube. In *Heptanchus* a corresponding thicker band runs on the frontal surface of the opticus. In *Squalus* the thickened wall lies dorsofrontally upon the opticus. In the presence of the thickened frontal part of the opticus tube in the selachians I recognize a primary phylogenetical stage repeated in the early ontogenetical development of the ligamentum in *Salmo*.

The mode of development of the "ligamentum" in *Salmo* and its dependence of the optic nerve also in Selachians demonstrates that it cannot be homologous with the eye-stalk in Selachians, where the latter develops from the premandibular somite (HOLMGREN, 1940).

LITERATUR QUOTED.

ALDINGER, H. 1937. Permische Ganoidfische aus Ostgrönland. København, Medd. om Grönland Bd. 102.

ALLIS, E. P. j:r. 1897. The cranial muscles and cranial first spinal nerves in Amia calva. Journ. Morph. 12.

— 1908. The pseudobranchial and carotid arteries in Amiurus. Anat. Anz. Bd. 33.

— 1909. The cranial anatomy of the mail-cheeked fishes. Zoologica Bd. 22, H. 57.

— 1912 a. The branchial, pseudobranchial and carotid arteries in *Heptanchus* (Notidanus) *cinereus*. Anat. Anz. Bd. 41.

— 1912 b. The branchial, pseudobranchial and carotid arteries in Raja radiata. Anat. Anz. 41.

— 1912 c. The pseudobranchial and carotid arteris in Esox, Salmo and Gadus. Anat. Anz. 41.

— 1914 a. Certain homologies of the palato-quadrate of Selachians. Anat. Anz. 45.

— 1914 b. The pituitary fossa and trigemino-facialis chamber in Selachians. Anat. Anz. 46.

— 1914 c. The pituitary fossa and trigemino-facialis chamber in Ceratodus forsteri. Anat. Anz. 46.

— 1915. The homologies of the hyomandibula of the gnathostome fishes. Journ. Morph. 26.

— 1918. On the origin of the hyomandibular of the teleostome. Anat. Rec. 15.

— 1919. The myodome and trigemino-facialis chamber of fishes, and the corresponding cavities in higher vertebrates. Journ. Morph. 32.

— 1922. The cranial anatomy of Polypterus, with special reference to *Polypterus bichir*. Journ. Anat. 56.

— 1923. Are the polar and trabecular cartilages of vertebrate embryo the pharyngeal elements of the mandibular and premandibular arches? Journ. Anat. 57.

— 1925. On the origin of the V-shaped branchial arch in the teleostomi. Proc. zool. soc. London 1925.

— 1928. Concerning the homologies of the hyomandibula and preoperculum. Journ. Anat. 62.

— 1931. Concerning the homologies of the hypophysial pit and the polar and trabecular cartilages. Journ. Anat. 65.

DE BEER, G. R. 1924 a. Studies on the vertebrate head. Part. I. Quart. Journ. micr. sci. 68.

— 1924 b. Contributions to the study of the development of the head in Heterodontus. Ibid.

— 1925. Contributions to the development of the skull in sturgeons. Quart. J. micr. sci 69.

— 1926. Studies on the vertebrate head. Part. II. Quart. J. micr. sci. 70.

— 1927. The early development of the chondrocranium of Salmo fario. Quart. J. micr. sci. 71.

— 1931 a. The development of the skull of Scyllium (Scylliorhinus) canicula L. Quart. J. micr. sci. 74.

— 1931 b. On the nature of the trabecula cranii. Quart. J. micr. sci. 74.

— 1937. The development of the vertebrate skull. Oxford.

DE BEER, G. R. and MOY, THOMAS, J. A. 1935. On the skull of Holocephali. Philos. Transact. Roy Soc. London, Ser. B. 224.

BOEKE. (1902). On the development of the entoderm etc. in Muraenoïds. Koningl. Akad. van Wetensch. Amsterdam.

BOYER, E. R. (1892). The mesoderm in Teleosts. Bull. Mus. Comp. Zool. Harvard College. 23.

VOL. 24 1943

Bridge, T. W. 1879. The osteology of Polyodon folium. Phil. Trans. Roy Soc. London. 169.

— 1888. Some points in the cranial anatomy of Polypterus. Proc. Philos. Soc. Birmingham. 6.

Bugajew, J. 1929. Über das Pharyngomandibulare der Knorpelganoiden. Anat. Anz. 67.

— 1930. Über den Bau des Oberkieferapparates bei den Acipenseroidei und den niederen Haifischen. Anat. Anz. 68.

Damas, H. 1935. Contribution à l'étude de la Métamorphose de la tête de la Lamproire. Trav. Inst. van Beneden, fascicule 27.

— 1943. Recherches sur le développement de *Lampetra fluviatilis L.* Thèse d'Agrég. Univ. de Liége.

Daniels, J. F. 1922. The elasmobranch fishes. Univ. Calif. Press.

Dohrn, A. (1884, 85). Studien zur Urgeschichte des Wirbeltierkörpers IV, VII, Mitteil. a. d. Stat. Neapel. 5, 6.

Dücker, M. 1924. Über die Augen der Zyklostomen. Jenaisch. Z. f. Nat. 60.

Edgeworth, F. H. 1926. On the hyomandibula of Selachii, Teleostomi and Ceratodus. Journ. Anat. 70.

— 1935. The cranial muscles of vertebrates. Cambridge Univ. Press.

Franz, V. 1932. Auge und Akkomodation von Petromyzon (Lampetra) fluviatilis. Zool. Jahrb. (Physiol.) 52.

— 1934. Höhere Sinnesorgane. Vergleichende Anatomie des Wirbeltierauges. Der Handb. d. vergl. Anat. der Wirbeltiere von Bolk, Göppert, Kallius und Lubosch. 2,2.

Fürbringer, K. 1903. Beiträge zur Kenntnis des Visceralskelets der Selachier. Morph. Jahrb. 31.

Fürbringer, M. (1903). Notiz über die oberflächliche Knorpelelemente im Kiemenskelete der Rochen (Extraseptalia) etc. Morph. Jahrb. 31.

Garman, S. 1913. The plagiostomia. Mem. Mus. Comp. Zool. Harvard. 34.

Gaupp, E. 1906. Die Entwicklung des Kopfskeletts. Hertwigs Handbuch der vergl. u. exper. Entwicklungslehre. Jena. 3(2).

Gegenbaur, K. 1872. Das Kopfskelet der Selachier. Untersuchungen zur vergleichenden Anatomie der Selachier. H. 3. Leipzig.

Goronowitsch. 1885. Studien über die Entwicklung des Medullarstranges bei Knochenfischen nebst Beobachtungen über die erste Anlage der Keimblätter und der Chorda bei Salmoniden. Morph. Jahrb. 10.

Gregory, W. K. 1904. The relations of the anterior visceral arches to the chondrocranium. Biol. Bull. 7. Woods Hole.

Haller, Graf. 1923. Über die Bildung der Hypophyse bei Selachiern. Morph. Jahrb. 53.

Hammarberg, F. 1937. Zur Kenntnis der ontogenetischen Entwicklung des Schädels von Lepidosteus platystomus. Acta Zool. 18.

Henneguy, L. F. 1888. Recherches sur le développement des poissons osseux. Journ. de l'anat. et phys. 24.

Hochstetter, F. (1888). Venensystem der Amphibien und Fische. Morph. Jahrb. 13.

— 1906. Die Entwicklung des Blutgefässystems. Hertwigs Handbuch der Entwicklungslehre. 6. Jena.

Hoffmann, L. 1913. Zur Kenntnis des Neurocraniums der Pristiden und Pristiophoriden. Zool. Jahrb. (Anat. u. Ontog.). 33.

Holmgren, N. 1940. Studies on the head of fishes. Part. I. Acta Zool. 21.

— 1941. Studies on the head of fishes. Part. II. Acta Zool. 22.

— 1942. Studies on the head of fishes. Part. III. Acta Zool. 23.

HOLMGREN, N. and STENSIÖ, E. 1936. Kranium und Visceralskelett der Acranier, Cyclostomen und Fische. In Bolk, Göppert, Kallius und Lubosch: Handb. der vergl. Anat. 4. Berlin und Wien.

HUBENDICK, B. 1942. Zur Kenntnis der Entwicklung des Primordialcraniums bei Leuciscus rutilus. Arkiv f. Zool. 34 A.

HUXLEY, T. H. 1876. On Cerotodus forsteri, with observations on the classification of fishes. Proc Zool. Soc. London.

IWANZOW, N. A. 1887. Der Scaphirhynchus. Bull. Soc. Imp. Nat. Moscou. 1.

JABLONOWSKI, J. 1898. Über einige Vorgänge in der Entwicklung des Salmonidenembryos. Anat. Anz. 14.

KOLTZOFF, 1902. Entwicklungsgeschichte des Kopfes von Petromyzon planeri. Bull. Soc. Imp. Nat. Moscou. 15.

KRIVETSKY, A. 1917. Sur la morphologie des élements de l'arc hyoïde ches les Sélaciens. Rev. Zool. russe. 2.

KUPFFER, C. VON. 1893. Studien zur vergleichenden Entwicklungsgeschichte des Kopfes der Kranioten. München und Leipzig.

LUTHER, A. 1909. Beiträge zur Kenntnis von Muskulatur und Skelett des Kopfes des Haies *Stegostoma tigrinum* Gm. und der Holocephalen. Acta soc. sci. fenn. 37.

MATVEIEV, B. 1925. The structure of the embryonal axial skull of the lower fishes. Bull. soc. nat. Moscou. 34.

MOY-THOMAS, J. A. 1933. Notes on the development of the chondrocranium of *Polypterus senegalus*. Quart. J. Micr. Soc. London. N.S. 76.

NEUMAYER, L. 1932. Studie über die Entwicklung des Kopfes von Acipenser. I. Acta Zool. 13.

NIELSEN, E. 1936. Some few preliminary remarks on Triassic fishes from East Greenland. Meddel. om Grønland. 112.

— 1942. Studies on Triassic fishes from East Greenland. I. Glaucolepis and Boreosomus. Palaeozoologica groenlandica. 1.

OSTROUMOFF, A. 1905. Zur Entwicklungsgeschichte des Sterlets (Acipenser ruthenus). Zool. Anz. 32.

PARKER, W. K. 1876. On the structure and development of the skull in sharks and skates. Tranz. Zool. Soc. 10.

PEHRSON, T. 1922. Some points in the cranial development of teleostomian fishes. Acta Zool. 3.

— 1940. The development of dermal bones in the skull of Amia calva. Acta Zool. 21.

POLLARD, H. B. 1891. On the anatomy and phylogenetic position of *Polypterus*. Anat. Anz. 6.

— 1892. On the anatomy and phylogenetic position of *Polypterus*. Zool. Jahrb. (Anat. u. Ontogen.). 5.

RABL, P. 1892. Über die Entwicklung des Venensystems der Selachier. Festschr. f. Leuckart.

REIS, O. M. 1896. Über Acanthodes Bronnii. Ag. Morph. Arb. v. G. Schwalbe. 6.

RIDEWOOD, W. G. 1896. On the spiracle and associated structures in elasmobranch fishes. Anat. Anz. 9.

ROMER, A. S. 1937. The braincase of the Carboniferous Crossopterygian Megalichthys nitidus. Bull. Mus. Comp. Zool. Harvard College. 82.

— 1941. Notes on the crossopterygian hyomandibular and braincase. Journ. of Morph. 69.

SAVATSKY, A. M. 1911. Die Entwicklung des larvalen Haftapparates beim Sterlet (Acipenser ruthenus). Anat. Anz. 40.

VOL. 24 1943

SCAMMON, R. E. 1911. Normal plates of the development of Squalus acanthias. Normentafel von Keibel. Jena.

SCHAUINSLAND, H. 1903. Beiträge zur Entwicklungsgeschichte und Anatomie der Wirbeltiere. Zoologica. 16.

SCHLEIP, W. 1904. Die Entwicklung der Kopfknochen bei dem Lachs und der Forelle. Anatomische Hefte. 23. (72. Heft).

SCHMALHAUSEN, J. J. 1923. Der Suspensorialapparat der Fische und das Problem der Gehörknöchelchen. Anat. Anz. 56.

SEWERTZOFF, A. N. 1916. Études sur l'évolution des Vertébrés inférieurs. 1. Arch. russ. d'Anat., d'Histol. et d'Embryol. I, 1.

— 1923. Die Morphologie des Visceralapparates der Elasmobranchier. Anat. Anz. 56.

— 1927. Études sur les vertébrés inférieurs. Publ. Staz. Zool. Napoli. 8.

— 1928. The head skeleton and muscles of Acipenser ruthenus. Acta Zool. 9.

SEWERTZOFF, A. N. and DISLER, N. N. 1924. Das Pharyngomandibulare der Selachier. Anat. Anz. 58.

SMITH, J. L. B. 1940. A living Coelacanthid fish from South Africa. Trans. Roy. Soc. of South Africa. 28.

STENSIÖ, 1921. Triassic fishes from Spitzbergen. I. Wienna 1921.

— 1925. Triassic fishes from Spitzbergen. II. K. Sv. Vet. Ak. Handl. Ser. 3. 2.

— 1927. The downtonian and devonian Vertebrates of Spitzbergen. I. Family Cephalaspidae. Skrifter om Svalbard. n:r 12.

— 1932. Triassic fishes from East Greenland. Meddel. om Grønland. 83.

— 1934. On the head of certain Arthrodires. K. Sv. Vet. Ak. Handl. 13.

STÖHR, P. 1892. Zur Entwicklungsgeschichte des Kopfskeletes der Teleostier. Festschr. der Julius-Maximilians-Univ. Würzburg. Leipzig.

SWAEN, A. and BRACHET, A. 1899. Étude sur les premières phases du développement des organes dérivés du mésoblaste chez les Poissons téléostéens. Arch. Biol. 16.

SWINNERTON, H. H. 1902. A contribution to the morphology of the teleostomian head skeleton... (Gasterosteus aculeatus). Quart. Journ. Micr. Sci. N. S. 45.

SÄVE-SÖDERBERGH, G. 1936. On the morphology of Triassic Stegocephalians from Spitzbergen, and the interpretation of the endocranium in the Labyrinthodontia. Sv. Vet. Ak. Handl. 16.

TRAQUAIR, R. H. 1877. The ganoid fishes of the British Carboniferous formations. I. Palaeoniscidae. Palaeontogr. Soc. 31.

— 1879. On the structure and affinities of the Platysomidae. Trans. Roy. Soc. Edinburgh. 29.

TRETJAKOFF, D. 1926. Das Auge der Plectognathen. Z. f. wiss. Zool. 127.

WATSON, D. M. S. 1925. The evolution and origin of the Amphibia. Phil. Trans. Roy. Soc. London. Ser. B. 214. 1926.

VETTER, B. 1874. Untersuchungen zur vergleichenden Anatomie der Kiemen- und Kiefer-Muskulatur der Fische. Jen. Zeitschr. 8.

WHITE, P. J. 1896. The existence of skeletal elements between the mandibular and hyoid arches in Hexanchus and Laemargus. Anat. Anz. 11.

VAN WIJHE, J. W. 1882. Über das Visceralskelett und die Nerven des Kopfes der Ganoiden und von Ceratodus. Niederl. Arch. f. Zool. 5.

— 1922. Frühe Entwicklungsstadien des Kopf- und Rumpf-Skeletts von Acanthias vulgaris. Bijdragen tot de dierkunde. 22.

WOSKOBOJNIKOW, M. 1914. Studien zur Kenntniss der Branchiomerie der Wirbeltiere. I—II. Mém. de la Soc. d. Natural. Kiew. (Russian.)

STUDIES ON THE HEAD OF FISHES

EXPLANATION OF LETTERINGS.

a, area of attachment of the first gill arch.
a.b.caps., anterior basicapsulur commissure.
a.c.ch., acrochordal tissue.
a.c.cm., groove and canal for the arteria carotis communis.
acr.+pb., acrochordal + polar cartilage.
acr.v.p., acrochordal vein plexus.
a.c.v., anterior cerebral vein.
a.gr., aortic groove.
ao., paired aorta.
a.o.c., antorbital cartilage.
ao.d., aorta dorsalis.
a.opht., arteria ophthalmica magna.
a.opt., arteria optica.
aort., aorta canal.
a. ps., notch for the efferent pseudobranchial artery.
art.sup.I., articulation of the suprapharyngobranchial I.
aud.c., auditory capsule.
aud.v., auditory vesicle.
bas.hy., basihyal.
bas.md., basimandibular.
b.p., basitrabecular process.
bp.tp., basitrabecular process.
br.ec., branchial ectoderme.
br.I., first branchial arch.
bsph.l., lateral basisphenoid rod.
bsph.m., medial basisphenoid rod.
bucc.VII., nervus buccalis VII.
caps.v., vein from outside of the auditory capsule.
car.com., carotis communis.
carot., carotis interna.
carot.ext., carotis externa.
cent., centrum for development of the extracarotid part of trabecle.
cer.hy., ceratohyale.
cer.md., ceratomandibulare.
cer.pl., cerebral vein plexus.
ch.pl., chorioid plexus.
c.i., carotis interna.
c.ju., posterior opening of the jugulo-hyomandibular canal.
com., premandibular commissure.
com.car., carotid commissure.
com.md.orb., communication between mandibular plexus and orbital sinus.
com.pq., palatoquadrate commissure.
con.h.v., connection of head vein and orbital sinus.
c.r., cartilage rod.
c. trh., jugulo-hyomandibular canal.
d.bl., dorsal blastema of the hyomandibula.
d.cuv., ductus cuvieri.
d.e.b.v., dorsal eye-brow vein.
d.end., ductus endolymphaticus.
dent.bl., dental blastema.
d.s., dorsum sellae.
Ec, ethmoidal bone mass.
eff.br.a., efferent branchial artery.
e.h.pl., external hyoid vein plexus.
e.hy., efferent hyoid artery.
e.m.mes., eye-muscle mesoderme.
e.m.v., eye-muscle vein.
ent., entoderme.
ep., articular process for autopalatinum (in fig. 48).
ep., epiphysis.
epa., efferent pseudobranchial artery.
e.V.pl., vein plexus outside the V-ganglion.
ep.br., epibranchial.
ep.hy., epihyal.
ep.md., epimandibular.
ep.v., epiphysial vein.
et.ha., ethmoid articulation of palatoquadrate.
et.p., ethmoidal plate.
et.v., vein from the ethmoidal region.
et.v.l., extratrigeminal vein loop.
ex.hm.pl., extrahyomandibular plexus.
ex.pq.pl., extra-palato-quadrate plexus.
ex.pq.v., extra-palato-quadrate vein.
ext.b.o.s., external branch from the orbital sinus.
eye., eye.
eye.v., eye vein.
f.b., fossa Bridgei.
f.b.ant., anterior part of the fossa Bridgei.
fen.opt., fenestra optica.
f.hm., articular fossa for hyomandibula.
f.hmd., dorsal articulation of the hyomandibula.

VOL. 24 1943

f.hmv., ventral articulation of the hyomandibula.
font., dorsal fontanella.
fr.mes., frontal mesoderme.
gj., jugular vein groove.
hm., hyomandibula.
h.mb., horizontal membrane.
hmd., dorsal facet for the hyomandibula.
hm.pr., hyomandibular process.
hm.s., horizontal myodomic septum.
hm.v., ventral facet for the hyomandibula.
h. r., hyomandibular ray.
hv., head vein.
hy.a., hyoid arch.
hy.c., hyoid constrictor muscle.
hyp., hypophysis.
hyp.hy., hypohyale.
hyp.md., hypomandibular.
ic., inner carotid.
i.f.v.l., intrafacial vein loop.
i.h.m.pl., internal hyoid and mandibular vein plexus.
i.md.pl., internal mandibular plexus.
int.b., intermediating body of palato-quadrate.
int.pq.pl., internal palato-quadrate plexus.
i.orb.s., interorbital septum.
i.o.s., infraorbital sinus. In fig. 69, interorbital septum.
i.o.sens., infraorbital sensory line or organ.
i.o.v., infraorbital vein.
i.ph.br., infra-pharyngo-branchial.
i.ph.md., infra-pharyngo-mandibular.
jca., anterior opening of the jugular canal.
jcp., posterior opening of the jugular canal.
jd., jugular depression.
jug.c., jugular canal.
K.c., cartilage of Källberg.
lam., connecting lamella between pq and trab.
lam.b.caps., lamina basicapsularis.
lat., foramen for lateralis nerve accompanying V.
l.bas., lamina basiotica.
l.c., lateral commissure.
lig., ligament (connecting pq. with trab.).
l.l.v., lateral line vein.
l.orb.s., lower orbital sinus.
l.orb.v., lower orbital vein.
m.a., mandibular arch.
max.V., n. maxillaris V.
m.b.c., medial band of cells.
Mc., Maeckel's cartilage.
md., mandible.
md.V., r. mandibularis V.
md.a., mandibular arch.
md.c., mandibular constrictor.
md.m., mandibular muscle.
md.r.(symp.), mandibular ray (symplectic).
md.s., mandibular somite.
md.s.v., vein from inside of mandibular somite.
mes.r., mesoderme ridge.
mes.v., mesencephalic vein.
m.o.i., musculus obliquus inferior.
m.o.s., musculus obliquus superior.
m.pr.hm., musculus protractor hyomandibulae.
m.pr.md., muscular process of somite II.
m.r.a., musculus rectus anterior (internus).
m.r.e., musculus rectus externus.
m.r.i., musculus rectus inferior.
m.r.s., musculus rectus superior.
mx., maxillary bone.
mx.bl., maxillary blastema.
my., myodome.
my.I., first myotome.
nas.pl., nasal placode.
nas.s., nasal sac.
n.ch., notochord.
n.cr.I., first neural crest.
n.cr.II., second neural crest.
OA., orbital artery.
o.n.c., orbito-nasal canal (or profundus canal).
o.n.v., orbito-nasal vein.
op.b., opercular blastema.
oph.a., ophthalmic artery.
ophma., arteria ophthalmica magna.
op.pr., opercular process.
op.s., nervus ophthalmicus superficialis VII.
opt., optic nerve.
opt.v., vein from eye.
op.v., opercular vein.
or.a., oral arch.
orb.bl., orbital cartilage blastema.
orb.c., orbital capsule.
orb.sin., orbital sinus.
pal., foramen for the palatine nerve.
pal.VII., nervus palatinus VII.

pal.p., palatine part.
pal.pl., palatine vein plexus.
pal.sin., palatine sinus.
pal.t., dermo-palatine tooth.
pal.v., palatine vein.
p.b., polar cartilage.
p.b.caps., posterior basicapsular commissure.
p.card., pericard.
p.ch., parachordal.
p.c.v., posterior cerebral vein.
ph.hy., pharyngo-hyal.
pit.com., pituitary commissure.
pit.v., pituitary vein.
pm.com., premandibular commissure.
pmx., premaxillary bone.
pmx.t., premaxillary teeth.
pm.v., premandibular somite.
pm.v.c., premandibular vein commissure.
p.occ., pila occipitalis.
PPAR., paroccipital or parotic process (ROMER).
p.par., posterior parachordal.
pq., palatoquadrate.
pq.ant., anterior part of palatoquadrate.
pq.pl., palato-quadrate vein plexus.
pr., postotic process.
pr.asc$_1$, *pr.asc*$_2$, anterior and posterior wing of parasphenoid bone.
pr.b., prootic bridge.
pr.ent., preoral entoderm.
prof.?, profundus nerve?
pr.o.s., primary orbital sinus.
pr.ot.e., processus oticus externus.
pr.ot.i., processus oticus internus.
pr.po., processus postorbitalis.
pr.pq., process of the palato-quadrate.
pr.trab., trabecular process.
p.s.b., efferent pseudobranchial artery.
p.s.c., posterior semicircular canal.
P.sph., *p.sph.*, parasphenoid bone.
rb.bl., ray bar blastema.
r.hy.VII., ramus hyoideus VII.
r.md.VII., ramus mandibularis VII.
r.o.p., canal for the ramus opercularis VII.
s.aort., groove for the radix aortae.
s.d., sucking disc.
sec.v., secondary vein.
s.hy.v., subhyomandibular vein.
s.o.a., subocular arch.
s.o.c., supraorbital cartilage.
s.oc.mes., subocular mesoderme.
som.I., premandibular somite.
som.II., mandibular somite.
som.III., hyoid somite.
s.o.s., supraorbital sinus.
s.o.sens., supraorbital sensory canal.
s.o.v., supraorbital vein.
s.ph.br., supra-pharyngo-branchial.
s.ph.md., supra-pharyngo-mandibular.
spic., spiracular canal.
spir., spiracle.
spir.a, spiracular area.
spir.c., spiracular cartilage.
spir.r., spiracular ray.
sp.occ., foramen for spino-occipital nerve.
st.hy., stylo-hyal.
s.tr.l., subtrabecular lamina.
sym.c., symphysial cartilage.
symp., symplectic.
t., cartilaginous tip of the epihyal.
tc., trabecle.
tent., tentacle rudiment.
tent.b., tentacle blastema.
tent.c., tentacle cartilage.
t.orb.c., thickened bar of the orbital capsule.
trab., trabecle.
trab.e., external part of trabecle.
trab.i., internal part of trabecle.
tr.con., transverse commissure.
tr.hm.VII., truncus hyomandibularis VII.
v.c.a., foramen for anterior cerebral vein.
v.c.d., foramen for vena capitis dorsalis.
v.c.int., vena cerebralis intermedia.
V.C.L., vena capitis lateralis.
v.c.l., vena capitis lateralis.
v.c.m., vena capitis medialis.
v.e.b.v., ventral eye-brow vein.
v.font., vestibular fontanella.
v.j., groove for vena jugularis.
vom.t., vomeral teath.
v.o.s., ventral orbital sinus.
v.pr., vein from the protractor hyomandibularis muscle.
v.s., vein sinus.
I., olfactory nerve.
II., optic nerve.
III., oculomotor nerve.
IV., trochlear nerve.

VOL. 24 1943

V., foramen for the trigeminal nerve.
V.mm., maxillary and mandibular branch of V.
V.p.+VI., profundus + abducens nerv.
VII., facialis nerve, foramen for.
VII.h., ramus hyoideus VII.
VII.lat., anterior lateralis nerve.
VII.M., ramus mandibularis VII.
VII.pal., ramus palatinus VII.
IX., nervus glossopharyngeus.
IX, d, d_1, d_2, canal for dorsal branches of IX.
IX.+fr., foramen for IX + fenestra rotunda?
IX.l., vein loop around the IX nerve (secondary vein-loop).
X., nervus vagus.
X.l., vein loop around the X nerve (secondary vein loop).

VOL.
24
1943

ÜBER DIE URSACHEN DER KOLLOIDALEN VERÄNDERUNGEN DES PROTOPLASMAS

UND DER ERHÖHTEN AFFINITÄT DES LETZTEREN DEN FARBSTOFFEN GEGENÜBER UNTER DEM EINFLUSSE SCHÄDIGENDER AGENTIEN

VON

W. J. ALEXANDROV und *D. N. NASSONOV*

(Aus dem cytologischen Laboratorium des Abteiles für allgemeine Morphologie. VIEM. Leningrad.)

Die Beobachtungen am Protoplasma lebendiger Zellen bei Einwirkung verschiedener schädigenden Agentien ist für die moderne Cytophysiologie von ausserordentlicher Wichtigkeit. An der Hand dieser Beobachtungen können die Forscher nicht nur den Mechanismus der Einwirkung dieses oder jenen Faktors studieren, sondern auch die Grundeigenschaften der lebenden Substanz erkennen. Gegenwärtig verfügen wir schon über ein reiches Tatsachenmaterial hinsichtlich der kolloiden und physikalisch-chemischen Umwandlungen des Protoplasmas der geschädigten Tier- und Pflanzenzellen. Es hat sich aber herausgestellt, dass trotz der grossen Mannigfaltigkeit der untersuchten Objekte und der Anwendung eines ganzen Arsenals vollkommen verschiedenartiger Einwirkungen, der Charakter der Veränderungen in der lebenden Substanz ausserordentlich eintönig ausfällt. Auf Grund der erhaltenen Tatsachen sind wir imstande für die kolloidalen und physikalisch-chemischen Veränderungen ein Schema zu entwerfen, welches für die Mehrzahl der Protoplasmabeschädigungen giltig ist. Dieses Schema lässt sich auf folgende Erscheinungen zurückführen: das grundlegende, die Beschädigung der Zelle stets begleitende Phänomen besteht in der Dispersionsverminderung der Protoplasma-Kolloide und in der Erhöhung der Plasmaviskosität. Die Dis-

persionsabnahme sowohl im Kern, als auch im Cytoplasma ist von zahlreichen Forschern auf Grund der Anwendung von Agentien festgestellt worden, die ihrer physikalischen und chemischen Eigenschaften nach vollkommen verschieden sind: mechanische Einwirkung, Wärme, ultraviolette Strahlen, Radium, Röntgenstrahlen, elektrischer Strom, Hypotonie, Säuren, Basen in schwachen Konzentrationen, verschiedene Salze, Narkotika, Alkaloiden und and. (Siehe Literatur: LEPESCHKIN 1937; NASSONOV und ALEXANDROV 1937; MAKAROV 1938.) Nicht weniger mannigfaltig und zahlreich sind die Agentien, welche die Protoplasmaviskosität erhöhen (Literatur siehe bei HEILBRUNN 1928). In vielen Fällen geht der Viskositätserhöhung ein gewisser Abfall der Viskosität voraus. Die Verminderung der Dispersion mit gleichzeitiger Viskositätserhöhung weist darauf hin, dass bei der Beschädigung des Protoplasmas eine Gelatinierung der Kolloide stattgefunden hat. Gleichzeitig beobachten wir im Protoplasma eine Koagulation und Koazervation der Kolloide. Dieselben finden ihren Ausdruck in der Fällung von Granula, der Entmischung von Vakuolen und dem Auftreten von Strukturen, die sonst in der intakten Zelle unsichtbar sind. Somit stellt die Dispersionsverminderung des Protoplasmas, welche im Resultat der Hydrophilieabnahme auftritt, eine eintönige, nicht spezifische Reaktion dar. In dieser Weise beantwortet die Zelle fast alle Beschädigungen. Wenn die Wirkung des alternierenden Agentien nicht zu weit vorgeschritten ist, erweist sich die Verschiebung des kolloidalen Zustandes als reversibel und die Zelle kehrt nach dem Einstellen der Einwirkung zur Norm zurück.

Es ist schon früher bekannt gewesen, dass bei der Beschädigung neben den kolloiden Veränderungen verschiedenartige Störungen in der normalen Verteilung der Substanzen zwischen der Zelle und der Umwelt stets stattfinden. Dieselben finden ihren Ausdruck einerseits in der erhöhten Aufnahme von Stoffen, welche unter normalen Verhältnissen in die Zelle nur sehr schwach oder überhaupt nicht eindringen, anderseits im Austritte aus der Zelle in die Umwelt einiger Stoffe, die von der unbeschädigten Zelle sonst festgehalten werden (z. B. die Hämolyse, in einigen Fällen — der Austritt von Pigment, der Austritt von Phosphaten (MAGAT 1936)).

Die Versuche mit der Vitalfärbung geben uns schöne und allbekannte Beispiele für die Störungen in der Art der Stoffverteilung bei schädigenden Einwirkungen. Bekanntlich lassen sich meistens die Kerne und das Cytoplasma der normalen Zellen von Vitalfarbstoffen nicht tingieren. Bei Färbung geschädigter Zellen wird hingehen stets eine mehr oder weniger intensive Anfärbung der ganzen Protoplasmamasse beobachtet.

Die Beschädigungen der Zelle rufen in der Hauptmasse des Protoplasmas ausserordentlich wichtige Umwandlungen hervor: das Studium des kolloidalen Zustandes lässt diesbezüglich keinerlei Zweifel aufkommen. Dennoch werden

die Störungen in der Verteilung der Stoffe zwischen der Zelle und der Umwelt und im Speziellen — das veränderte Verhalten der Zelle den Vitalfarbstoffen gegenüber — oft grundverschieden gedeutet. Die Anhänger der Membrantheorie z. B. betrachten diese Störungen als Ausdruck der Veränderungen nur in der Oberflächenschicht der Zelle — der Zellmembran, welcher bei der Verteilung der Stoffe eine ausschlaggebende Rolle zugeschrieben wird. Diesem Standpunkte gemäss können die auf diesem Gebiete gemachten Beobachtungen für die Beurteilung der im Zellplasma selbst sich abspielenden Vorgänge keineswegs benutzt werden. Allein eine Reihe von Untersuchungen hat die Unhaltbarkeit der Membrankonzeption bewiesen und an vielen Beispielen gezeigt, dass die Eigenschaften der ganzen Protoplasmamasse die Verteilung der Stoffe zwischen Zelle und Umwelt entscheiden. (Moor u. Roaf 1913; Lapicque 1925; Fischer 1927; Bigvood 1927; Lepeschkin 1936; Styles 1937; Nassonov 1938, 1939; Aisenberg 1939; Alexandrov 1934, 1939; Nassonov u. Aisenberg 1937; Kamnev 1938; Troschin 1939; Utkina 1941 u. and.)

VOL. 24 1943

Mit besonderem Nachdruck wollen wir hervorheben, dass die Membrantheorie nicht imstande ist Ergebnisse der Vitalfärbung sowohl normaler, als auch beschädigter Zellen zu erklären. Die Richtigkeit dieser Behauptung haben wir experimentell schon in einer ganzen Reihe früher erschienener Arbeiten bewiesen (Nassonov 1930; Alexandrov 1932; Nassonov u. Alexandrov 1934, 1938).

Diese Untersuchungen haben ausserdem gezeigt, dass die infolge der Beschädigung der Zelle eintretende Färbung des Protoplasmas und des Kernes unmittelbar auf die erhöhte Fähigkeit der lebendigen Substanz den Farbstoff zu binden hinweist. Bei entsprechender Dosierung des Beschädigungsfaktors sieht man, dass die Veränderungen der Adsorptionsfähigkeiten des Protoplasmas — den kolloidalen Umwandlungen ähnlich — durchaus reversibel sein können. Die Untersuchungen von Nassonov 1930, 1932 und seinen Mitarbeitern (Alexandrov 1932; Makarov 1934, 1935, 1936, 1938; Kamnev 1934, 1936; Sutulov 1937; Braun u. Ivanov 1933; Aisenberg 1934) haben eine reversible Tinktion der lebendigen Substanz bei reparierbaren Einwirkungen an folgenden Objekten erhalten: *Frosch:* Epithelzellen des Darmes, der Haut, der Speiseröhre, der Harnblase, der Hornhaut; Sehnenzellen, Zellen des hyalinen Knorpels, des Mesothels; Zellen der glatten und quergestreiften Muskulatur; sympatische Nervenzellen des Herzens und der Harnblase; Zellen der Rückenmarkganglien; *Triton:* Zellen des Darmepithels und der quergestreiften Muskulatur; *Chironomus-Larve:* Zellen der Speicheldrüse, Epithel des Magens und Darmes, der Malpighischen Gefässe, der quergestreiften Muskulatur; *Daphnia;* Epipoditenzellen, Zellen des Fettkörpers und Eizellen; *Verschiedene Infusorien;* ausserdem sympatische, kom-

missurale, motorische und sensorische Nervenzellen bei einer Reihe von Mollusken, Coelenteraten, Würmer, Arthropoden und Tunikaten. Schliesslich hat SMOILOWSKAJA 1938 dasselbe Resultat bei Säugetieren eingehend studiert. Benutzt wurden: Sauerstoffmangel, Säuren, Basen, Methyl-Aethyl-Propyl-, Butyl-Amyl-Alkohol, Aceton, Æther, Chloroform, Chloralhydrat, CO; H_2S, Hypotonie, hohe Temperatur. Diese Tatsachen können noch durch zerstreute Angaben älterer und neuerer Autoren rücksichtlich der reversiblen Färbbarkeit des Protoplasmas erläutert werden (Literatur bei ALEXANDROV 1932; BECKER 1936; RIES 1937).

Somit bezeugen alle bekannten Untersuchungen, dass die lebendige Substanz die beschädigenden Einwirkung der Agentien der aller verschiedensten Natur stets mit einem einsinnigen und nicht spezifischen Veränderungskomplex beantwortet. Das primäre, noch reversible Stadium dieser Umwandelungen haben wir mit dem Namen „Paranekrose" belegt (NASSONOV u. ALEXANDROV 1934). Bei erhöhter Einwirkung geht der paranekrotische Zustand in das irreversible Stadium über, welches den Tod der Zelle herbeiführt. Bei diesem Übergange machen sich nur eine Verstärkung und eine fortschreitende Entwicklung der während des paranekrotischen Stadium beobachteten Substanzumbildungen kenntlich.

Die Liste der nicht spezifischen Vorgänge, welche die Beschädigung der Zelle begleiten, ist mit dem Hinweise auf die kolloidalen Umbildungen und die Adsorbtionsveränderungen wahrscheinlich noch nicht erschöpft. Viele Autoren beschreiben noch eine Verschiebung der interzellulären Reaktion in die saure Seite. Ausserdem wird in der Literatur das Auftreten anderer Verschiebungen im Inneren der geschädigten Zelle erwähnt: Lipophanerose, erhöhte Fluoreszenz, Veränderung im Absorbtionsspektrum u. s. w. Diese Angaben sind aber noch ungenügend und geben uns nicht das Recht die genannten Merkmale in die Charakteristik der Paranekrose einzuschliessen. Endlich haben wir alle Berechtigung anzunehmen, dass die Elektronegativität des verletzten Bezirkes zu den obligaten Merkmalen des paranekrotischen Zustandes gehört.

Alle uns zur Verfügung stehenden Kenntnisse der Veränderungen der lebendigen Substanz bei dem Übergange derselben in den toten Zustand genügen um die Frage über die Ursachen dieses Prozesses aufwerfen zu können. Die Lösung dieser Frage ist für das Verstehen der Eigenschaften des lebendigen Protoplasmas von grundsetzlicher Bedeutung. Gegenwärtig haben nur zwei Theorien es versucht eine zusammenfassende Antwort auf die gestellte Frage zu geben. HEILBRUNN(1928, 1930, 1932) und seine Schüler haben im Laufe mehrerer Jahre die Veränderungen in der Viskosität des Protoplasmas unter der Einwirkung mannigfaltigster Faktoren untersucht. HEILBRUNN nimmt an, dass die in der beschädigten Zelle sich abspielenden Umwandlungen der Gerinnung des Blutes vollkommen analog seien. Die

Plasmakoagulation oder die „Reaction of surface precipitation" stellt nach diesem Autor einen fermentativen Vorgang vor, bei welchem das Calciumion eine ausserordentlich wichtige Rolle spielt. HEILBRUNN versucht in dieser Weise die kolloidalen Veränderungen des Protoplasmas zu erklären, lässt aber die Frage über das veränderte Verhalten der lebendigen Substanz den vitalen Farbstoffen gegenüber unberührt.

Die Zweite, von LEPESCHKIN (1937) aufgestellte Hypothese beschränkt sich nicht nur auf die kolloidalen Umwandlungen, sondern ist bestrebt ein bedeutend weiteres Gebiet zu umfassen. Bei der Auseinandersetzung seiner Beschädigungstheorie geht der Forscher von Vorstellungen aus, die sich von den allgemein anerkannten Begriffen über den Bau der lebendigen Substanz kardinal unterscheiden. Nach LEPESCHKIN ist nämlich nicht das Wasser, sondern ein besonderes Lipoproteinkomplex das Dispersionsmittel des Protoplasmas. Dieses stellt eine ausserordentlich lockere Verbindung von Eiweiss und Lipoid dar und zerfällt infolge beliebiger Beschädigungen der Zelle. Im Resultat werden das freie Eiweiss und das Lipoid — welche in der intakten Zelle fehlen — befreit. Das in Gestalt von Tröpfchen entmischte Lipoid ergibt denn das Bild einer sogenannten Lipophanerose. Die Unfähigkeit des Protoplasmas der ungeschädigten Zellen sich zu färben erklärt LEPESCHKIN durch das Fehlen des freien Eiweisskörpers. Nach der Meinung des Autors fehlt dem an das Lipoproteinkomplex gebundenen Eiweiss die Fähigkeit sich mit dem Farbstoff zu binden, deshalb erfolgt die Cytoplasma- und Kerntinktion erst nachdem der Lipoproteinkomplex durch die Einwirkung des alternierenden Faktors zerfallen ist und das freie Eiweiss sich abgespaltet hat.

Ohne in die Erörterung der genannten Theorien als solche näher einzugehen wollen wir erwähnen, dass eine umfassende Beschädigungslehre eine Erklärung nicht nur der kolloiden Umwandlungen sondern auch der Adsorbtionsveränderungen des alterierten Protoplasmas geben muss. Davon ausgehend können wir wohl kaum die Ansichten von HEILBRUNN oder LEPESCHKIN teilen. Der erste Forscher umgeht einfach die Frage über die veränderten Affinität des Protoplasmas zu den Farbstoffen; LEPESCHKINS Deutung ist nicht genug begründet. Tatsächlich führt dieser Forscher eigentlich keinerlei Beweise zugunsten seiner Behauptung an, das Ausbleiben der vitaler Färbung des intakten Protoplasmas hänge von der Unfähigkeit des mit dem Lipoid gebundenen Eiweisses den Farbstoff aufzunehmen ab. Nun hat LIEBERMANN (1891) gezeigt, dass die Verbindungen des Eiweisses mit den Lipoiden, Lecitin-Albumin sich gerade sehr intensiv mit Methylenblau und anderen basischen Farben tingieren lassen. Auch in der speziell der Vitalfärbung gewidmeten Literatur finden wir keinerlei genügende Erklärungen für das Auftreten von Farbe im geschädigten Protoplasma. MÖLLENDORFF (1920,1921) und WANKEL (1921) sehen den Grund für das Fehlen einer wahrnehmbaren Tinktion des intakten Protoplasmas in der intensiven Reduktion des in der Zelle tatsächlich

VOL. 24 1943

vorhandenen Farbstoffes bis zu seiner Leukoform. Die Tinktion tritt erst ein wenn die Lebenskräfte der Zelle erschöpft sind und die Reduktion nicht mehr möglich ist. Die Autoren geben aber keine überzeugenden Beweise für diese trotz der Anwesenheit von Sauerstoff sich so energisch abspielenden Farbstoffreduktion. Die dargelegte Erklärungsweise erregt besondere Zweifel, wenn wir uns an folgenden Umstand erinnern: das Protoplasma der intakten Zelle bleibt an der Luft bei Anwendung des Neutralrots ungefärbt. Der E_0-Wert desselben liegt unter dem Oxyreduktionspotential selbst der in anaeroben Bedingungen befindlichen Zelle.

RIESEL (1930) stützt sich auf FEULGENS (1912, 1913) Versuche über die chemische Bindung der Nukleoproteide mit den basischen Farbkörpern und gibt folgende Erklärung für die Kerntinktion der geschädigten Zelle: infolge der Schädigung produziert die Zelle eine Säure, welche im Kerne das Freiwerden der Nukleinsäure hervorruft; letztere tritt mit dem Farbstoff in chemische Verbindung und bedingt die Anfärbung der Kernstrukturen. In den Kernen intakter Zellen fehlt die saure Reaktion, die Nukleinsäure ist an das Protein gebunden und die für die Tinktion notwendigen Bedingungen bleiben aus. Diese Deutung ist nach zwei Richtungen hin zweifelhaft: erstens wird eine Verstärkung der Tinktion nicht nur an Nukleoproteide enthaltenden Strukturen beobachtet, sondern auch — und mitunter sogar stärker und demonstrativer — an Zellkomponenten, welche laut der Feulgen'schen Reaktion keine Spur von Nukleinsäure aufweisen (Cytoplasma, wahre Nukleolen); zweitens bleibt es unerklärt weshalb bei der Beschädigung der Kerne und des Protoplasmas sich die Letzteren mit sauren Farbstoffen stärker tingieren lassen. (In Pflanzenzellen: HECHT 1912; GICKLHORN 1914; SCARTH 1926; KÜSTER 1926; PALTAUF 1928; KELLER 1932; in Tierzellen: SCHMIDT 1921; BALL 1927; WALLBACH 1931; HOFFMANN 1932; SOLITERMAN 1935.)[1]

In der letzten Zeit hat schliesslich RIES (1937) einen Versuch gemacht eine neue Erklärung zu geben. Er bestätigt die Richtigkeit unserer Daten in Betreff der erhöhten Farbstoffaffinität des geschädigten Protoplasmas und nimmt an, dass das Hindernis für die Tinktion des intakten Protoplasmas in der starken Hydration seiner Micellen liege. Bei verschiedenartigen Beschädigungen sinkt die Hydrophilie der Zellkolloide; letztere koagulieren und werden infolge der Abnahme der Solvathülle fähig die Farbkörper zu binden. Zu Gunsten seiner Auffassung führt RIES nur wenige Modellversuche an, welche an Eiweiss gemacht worden sind und einen vorläufigen Charakter tragen.

[1] In unseren früheren Arbeiten über die Wirkung der Vitalfarbstoffe haben wir NASSONOV (1930, 1932) und ALEXANDROV (1932) den Grund für die diffuse Färbung und die Gelatinierung des Protoplasmas und des Kernes in der Verschiebung der intrazellulären Reaktion in die saure Seite gesehen; jedoch in unseren späteren Schriften waren wir gezwungen unsere Ansicht zu widerrufen (siehe NASSONOV und ALEXANDROV (1934, 1938)).

Wie wir sehen sind die obenerwähnten Versuche nicht imstande die erhöhte Vitalfärbung des geschädigten Protoplasma befriedigend zu erklären. Für die Lösung des Beschädigungsproblems als Ganzes ist aber die richtige Vorstellung von den färberischen Fähigkeiten der lebendigen Substanz von allergrösster Wichtigkeit. Nachdem wir uns davon überzeugt hatten, dass die Beschädigung des Protoplasmas stets von einer erhöhten Farbstoff-Adsorbtionsfähigkeit begleitet wird, waren wir gezwungen unsere Aufmerksamkeit auf die Klärung der Gründe dieser Erscheinung zu richten. Wir hatten unsere Untersuchung mehrere Jahre vor dem Erscheinen der RIES'schen Arbeit begonnen. Wir sind ebenfalls ausgegangen von den bereits bekannten kolloidalen Veränderungen, welche sich im Protoplasma der beschädigten Zellen abspielen. Wie erwähnt, sind diese Umbildungen auf den Verlust der Hydrophilie der Protoplasmamicellen zurückzuführen, welche eine Schwächung ihrer Dispersion infolge beliebiger Alteration herbeiführt. Ausser den nativen Eiweisslösungen kennen wir eigentlich kein einziges Kolloid, welches ebenso einsinnig auf die Einwirkung gänzlich verschiedener physikalischer und chemischer Faktoren reagiert. Dagegen ist es in nativen Eiweisslösungen möglich eine Verminderung des Dispersiongrades und in den meisten Fällen sogar eine totale Fällung fast mithilfe eines jeden Faktors der auch die lebendige Zelle beschädigt, hervorzurufen. Wie im Protoplasma der Zelle, so auch in der Eiweisslösung sind diese Dispersionsveränderungen von einer Steigerung der Viskosität begleitet. Bei derartigen Einwirkungen auf die Eiweisslösung spielen sich gewöhnlich nicht nur kolloidale Umwandlungen ab, es können auch tiefe, oft irreversible Störungen der Eiweissmoleküle eintreten. Diese Eiweissveränderung führt bekanntlich den Namen „Denaturation".

Der Umstand, dass das native Eiweiss im Reagenzglase auf die verschiedenartigsten Einwirkungen mit denselben eintönigen kolloidalen Veränderungen reagiert wie das Protoplasma der lebendigen Zelle, zwingt uns die Frage aufzuwerfen, ob vielleicht auch den in der Zelle vorkommenden Veränderungen des Protoplasmaeiweisses Umwandlungen zu Grunde liegen, die denen des in vitro untersuchten nativen Eiweisses ähnlich sind? Sollte es uns gelingen dieses zu beweisen, so würden wir imstande sein das Beschädigungsproblem der Zelle wenn auch nur teilweise auf einem primitiveren und für das Experimentieren bequemeren System zu studieren. Im Zusammenhange damit haben wir zu klären wie weit der Parallelismus der im beschädigten Protoplasma sich abspielenden Prozesse und Vorgänge, die bei ähnlichen Einwirkungen die Denaturierung der Eiweisslösung begleiten, reicht. Im Speziellen müssen wir wissen, ob im nativen Eiwess während seiner „Schädigung" eine gleiche Verstärkung der Farbstoffaffinität erfolgt wie im alterierten Protoplasma. Der Lösung dieser Frage sind die dargelegten experimentellen Untersuchungen gewidmet.

VOL. 24 1943

Wir benutzen für unsere Untersuchungen Pferdeserum und Hühnereiweiss. Die ersten Versuche waren von provisorischem Charakter und wurden nach der BECHHOLD'schen Methode (1907) gestellt. BECHHOLD demonstrierte die Bindung des Methylenblaus durch die Serumeiweisse in folgender Weise: gleiche Quantitäten von Wolle wurden in dem einen Falle in einer Methylenblaulösung in dest. Wasser gefärbt, im anderen — in einer Lösung von gleicher, doch im dialysierten Blutserum angefertigten, Farbstoffkonzentration tingiert. Im Resultat war die Wolle aus der wässrigen Farblösung stärker gefärbt, als die Wolle, welche im Serum gelegen hatte. Daraus muss gefolgert werden, dass im Serum ein Teil des Farbstoffes im gebundenen Zustande verweilte. In unseren Versuchen zerschnitten wir ein kreisrundes aschefreies Filtrirpapier in zwei glechgrosse Teile. Die eine Hälfte gelang alsdann für 5 bis 10 Minuten in eine 0,1 %-ige Methylenblaulösung in nativem Serum. Die andere Hälfte dieses Filtrierpapieres wurde während derselben Zeitspanne in einer Methylenblaulösung in einen Serum gefärbt, welches vorher durch Erwärmen denaturiert worden war. Nach der eingetretenen Tinktion wurden beide Filter in Wasser gespült und in gleiche Volumen von 96°-igem Alkohol mit 2 %-igem Schwefelsäuregehalt gebracht. Der angesäuerte Alkohol extrahiert fast den ganzen Farbstoff, der vom Filtrierpapier adsorbiert war. Die Intensität der Tinktion dieser Alkoholextraktionen wurde mithilfe des PULFRICH'schen Stufenphotometers bestimmt. Der Quantität des Methylenblaues, welches von dem im nativen Serum gefärbten Papierblättchen adsorbiert war, haben wir den 100-Wert zugeschrieben; die Quantität des Farbstoffes, welcher die zweite Hälfte des Filtrierpapieres im denaturierten Serum gebunden hatte, wurde in %% des ersten Wertes ausgedrückt. In allen Fällen hatte die Hälfte aus dem denaturierten Serum weniger Farbe adsorbiert als die andere. Dieses Ergebnis kann in dem Sinn erklärt werden, dass die Eiweisse des denaturierten Serums mehr Farbstoff gebunden hatten als die nativen Eiweisse. Das dargelegte Verfahren erlaubt uns selbst bei ganz unbedeutenden Denaturationsgraden die erhöhte Bindefähigkeit des Eiweisses deutlich in Erscheinung treten zu lassen. Auf Tafel I sehen wir, dass das Serum nach einem 30 Minuten andauernden Erwärmen das Methylenblau merkbar stärker bindet als das native Serum. Infolgedessen ist die Färbung des in diesem Serum tingierten Filtrierpapiers um 20—30 % schwächer.

Ein klares Bild von der erhöhten Methylenblauaffinität des denaturierten Eiweisses können wir auch bei Anwendung einer anderen Versuchsmethode erhalten. Ein kreisrundes Filtrierpapierblättchen wurde 3 Minuten in einer 0,1 %-igen wässerigen Methylenblaulösung tingiert, während 2 Stunden in 3 Portionen reinen Wassers gespühlt und danach genau halbiert. Die eine Hälfte gelangt in ein natives Pferdeserum, die zweite Hälfte — in ein gleiches Quantum von Serum, welches aber vorher durch Erwärmen denaturiert war. Infolge der Desorbtion trat eine partielle Abfärbung des Filtrierpapiers ein.

Tabelle I.

Färbung des Filtrierpapiers mit Methylenblau, welches in nativem und denaturierten Serum gelöst war.		
Farbedauer	Denaturationstemperatur	Farbeintensität
5 Min.	57°	70 %
	57°	75 %
	Natives Serum	100 %
10 Min.	57°	79 %
	57°	79 %
	Natives Serum	100 %

Tabelle II.

Färbung des Filtrierpapiers in nativem und denaturierten Serum.	
Denaturationstemperatur	Quantität des Farbstoffrestes
71°	49 %
66°	85 %
57°	93 %
Natives Serum	100 %

VOL. 24 1943

Nach einer 30 Minuten andauernden Abfärbung wurden beide Hälften im Wasser gewaschen und in angesäuerten Alkohol weiter abgefärbt. Mittels der Kolorimetrierung der Alkoholextraktion konnte die relative Methylenblauquantität, welche in den beiden Hälften zurückgeblieben war, festgesetzt werden. Diese Versuche haben gezeigt, dass die Entfärbung im denaturierten Serum intensiver verläuft als im nativen. Dieses kann durch die stärkere Affinität des denaturierten Eiweisses zu den Farbkörpern erklärt werden. Auf Tabelle II sind die relativen Mengen des Methylenblaus gegeben, welche nach der Abfärbung eines Papiers im denaturierten Serum bei 3 Wärmegraden zurückgeblieben war. Auch hier entspricht der 100-Wert derjenigen Farbstoffquantität in der Kontrollhälfte, welche im nativen Eiweiss gelegen hatte. Wie aus der Tabelle zu ersehen ist, verläuft die Entfärbung umso energischer je stärker das Serum denaturiert war. Obgleich die Empfindlichkeit dieses Verfahrens der der obenerwähnten Methode nachsteht, sind die Ergebnisse doch genügend sicher. Zu dessen Gunsten sprechen die Kontrollversuche, bei welchen beide Filterhälften in nativem Serum entfärbt wurden. Die Extraktionen aus diesen unter gleichen Bedingungen entfärbten Hälften erwiesen sich der Intensität ihrer Tinktion nach als äusserst ähnlich und der Unterschied betrug nur wenige Prozente.

Somit zeigen schon die dargelegten Versuche, dass in Gemischen von Methylenblau mit nativem und denaturierten Serum, das durch Erwärmen denaturierte Eiweiss mehr Farbstoff bindet als das native. Die erhöhte bindefähigkeit des Eiweisses steigt mit dem Denaturationsgrad.

Nach diesen vorläufigen Versuchen sind wir an Experimente geschritten, die es uns ermöglichen die Farbstoffadsorbtion in Eiweisslösungen quantitativ genauer zu studieren. Der Sinn dieser Versuche liegt in der Feststellung der

Tinktionsintensität der nativen und denaturierten Eiweisslösungen, welche von der Farbstofflösung durch eine für den Farbstoff permeable, für die Eiweisspartikel aber undurchdringliche Membran getrennt waren.

Die Versuche wurden folgendermassen ausgeführt. Es wurden lange 8 mm breite Glassröhren mit Kollodium gefüllt. Danach wurde das Kollodium ausgegossen und die der Wand anhaftende Kollodiumschicht unter ununterbrochenem Drehen der Röhre ausgetrocknet. Sodann zogen wir die Kolloidhülse aus der Röhre heraus, füllten sie mit 5 ccm nativen oder denaturierten Eiweiss, banden beide Enden sorgfältig zu und brachten die Hülse in eine in Ringersalzlösung angefertigte Farbstofflösung. Nach der erfolgten Tinktion wurde die Farbstoffkonzentration im Innern der Hülse festgestellt. Da die unmittelbare Kolorimetrierung des aus der Hülse entnommenen Eiweisses infolge der Trübung der denaturierten Portion erschwert war, musste zu folgenden Manipulationen gegriffen werden. Hatten wir es mit basischem Farbstoffen zu tun, so entnahmen wir der Hülse genau 2 ccm der Eiweisslösung und setzten 5 ccm angesäuerten Alkohols hinzu. Einige Stunden nach der Fällung des Eiweisses war der Farbstoff durch die Alkohollösung vollkommen extrahiert. Letztere wurde nun filtriert[1] und im Pulfrich-Photometer nach seiner Farbstoffkonzentration geprüft. Bei Anwendung saurer Farbstoffe musste beim Kolorimetrieren ein anderes Verfahren angewandt werden da der angesäuerte Alkohol saure Farbstoffe nur mangelhaft extrahiert. Zu den 2 ccm des mit saurer Farbe tingierten Eiweisses setzten wir 5 ccm einer 1 %-igen KOH-Lösung hinzu. Nach mehreren Minuten ergab sowohl das native als auch das denaturierte Eiweiss eine ganz klare, für das Kolorimetrieren durchaus brauchbare Lösung.

Da für den Vergleich der Adsorbtionsbildungsintensität des nativen und denaturierten Eiweisses die absoluten Werte von keinerlei Bedeutung sind, konnten wir als relative Messeinheit den E-Wert (Extinktionskoeffizient) anwenden, der auf der Trommel des Photometers unmittelbar abgelesen wird und der Farbstoffkonzentration in der kolorimetrierten Lösung proportional ist. Die Färbung der Hülsen dauerte 3—4 Tage. Diese Dauer war auf Grund vorhergehender Versuche festgestellt worden, die gezeigt hatten, dass in dieser Zeitspanne zwischen der Lösung ausserhalb der Hülse und im Innern der selben das Gleichgewicht eintritt. Die Farbstoffkonzentration in der Hülse setzt sich aus der Menge des mit dem Eiweiss gebundenen und des frei im gelösten Zustande sich befindenden Farbstoffes zusammen. Deshalb wurde der E-Wert für die Lösung des denaturierten Eiweisses (E_d), für das native Eiweiss (E_n) und für die umgebende Lösung (E_l) bestimmt.[2]

[1] Der Filter blieb dabei farblos.

[2] Zwecks Erhaltung des E_l-Wertes wurde die umgebende Lösung ebenso verdünnt wie wie der Hülseninhalt bei der Feststellung des E_d- und E_n-Wertes.

Aus der Annahme ausgehend, dass bei eingetretenem Gleichgewichte die Konzentration des im Innern der Hülse frei gelösten Farbstoffes dem E_l-Werte sehr nahe liegen muss, konnten wir die Konzentration des mit dem denaturierten Eiweiss gebundenen Farbstoffes mit $E_d—E_l$ und die Konzentration des mit dem nativen Eiweiss gebundenen Farbstoffes mit $E_n—E_l$ bezeichnen.[1] Sollte die Eiweisskonzentration in beiden Lösungen gleich stark sein, so würde das Verhältnis $\frac{E_d - E_l}{E_n - E_l}$ uns die Beziehung zwischen den Grössen der Adsorbtionsbindung des denaturierten und des nativen Eiweisses wiedergeben. Allein die Beobachtungen haben gezeigt, dass diejenigen Hülsen, welche für unsere Farbstoffe gut durchlässig sind, den Austritt eines Teiles des Eiweisses nicht verhüten können.

Dieser Austritt ist am bedeutensten beim nativen Eiweiss; das denaturierte Eiweiss tritt umsoweniger aus, je stärker es denaturiert worden war. Infolgedessen war eine entsprechende Korrektion nötig. Zu diesem Zwecke wurde nach der Entleerung der Hülse der Prozentsatz des trockenen Eiweissrestes festgestellt.

VOL. 24 1943

Nach dem Substrahieren der Salze erhielten wir den %-Wert des trocknen Eiweisses in der nativen (T_n) und denaturierten (T_d) lösung. Somit geben die Verhältnisse $\frac{E_d—E_l}{T_d}$ und $\frac{E_n—E_l}{T_n}$ die Konzentrationen des in der 1 %-Lösung des denaturierten und nativen Eiweisses in relativen Einheiten wieder. Das Verhältnis der Farbstoffkonzentrationen zu den gleiche Eiweissmengen enthaltenen Lösungen zeigt gleichzeitig das Verhältnis der Farbstoffmengen, die durch die Eiweisse dieser Lösungen adsorbiert werden. Wenn wir die vom nativen Eiweiss gebundene Farbstoffquantität mit 100 bezeichnen, kann die durch das denaturierte Eiweiss gebundene Farbstoffmenge in %% von dieser Grösse ausgedrückt werden. Um den Einfluss dieses oder jenen denaturierenden Agentien auf die Bindefähigkeit des Eiweisses zu messen, werden wir weiterhin vorwiegend diesen Wert $\frac{(E_d - E_l) \cdot T_n \cdot 100}{(E_n - E_l)\ T_d} = X$ benutzen.

Zum Vergleich der Sorbtionsfähigkeit des denaturierten und nativen Eiweisses wurden 2 Hülsen gebraucht, welche aus den Nachbarbezirken ein und derselben Kollodiumröhre verfertigt waren. Beide Hülsen wurden in strenggleichen Bedingungen gefärbt. In allen Fällen erfolgte die Färbung im Dunkeln.

Mit Hilfe des dargelegten Verfahrens waren am genauesten die durch Wärmedenaturation hervorgerufenen Veränderungen der Sorbtionsfähigkei-

[1] Da die Lösung eine bedeutende Salzmenge enthielt war der Unterschied in der Konzentration des frei gelösten Farbstoffes zu beiden Seiten der Membran — trotz des Donnongleichgewichts von keiner besonderen Bedeutung.

Tabelle III.

Wirkung der Hitzedenaturation auf die Methylenblauadsorbtion (0,001 %) durch Eiweisse des Pferdeserums.

Hülsennummer	Hülseninhalt	Trocknes Eiweiss im %: T_d und T_n	Gesamtkonzentration des Farbstoffes: E_d und E_n; $E_l = 86$	Konzentration des gebundenen Farbstoffes. $(E_d - E_l)$ und $(E_n - E_l)$	Konzentration des gebundenen Farbstoffes in 1 %-ger Eiweisslösung: $\frac{E_d - E_l}{T_d}$ und $\frac{E_n - E_l}{T_n}$	Quantität des am denaturierten Eiweiss gebundenen Farbstoffes in Prozenten von der Menge des am nativen Eiweiss gebundenen: $X = \frac{(E_d - E_l) \cdot T_n \cdot 100}{(E_n - E_l) \cdot T_d}$	Der X-Mittelwert
1 a	Denat. bei 56°	5,02	187	101	20,1	209	187
1 b	Nativ	4,70	131	45	9,6		
2 a	Denat. bei 56°	5,72	201	115	20,1	166	
2 b	Nativ	5,47	152	66	12,1		
3 a	Denat. bei 62°	6,21	280	194	31,2	260	248
3 b	Nativ	4,51	140	54	12,0		
4 a	Denat. bei 62°	6,19	260	174	28,1	228	
4 b	Nativ	5,20	150	64	12,3		
5 a	Denat. bei 62°	6,21	284	198	31,9	242	
5 b	Nativ	4,99	152	66	13,2		
6 a	Denat. bei 62°	5,87	297	211	35,9	262	
6 b	Nativ	4,30	145	59	13,7		
7 a	Denat. bei 68°	6,14	492	406	66,1	555	555
7 b	Nativ	4,79	143	57	11,9		

Tabelle IV.

Wirkung der Hitzedenaturation auf die Methylenblauadsorbtion (0,001 %) durch Eiweisse des Pferdeserums.

Farbstoffadsorbtionswert X	Natives Serum	Denaturiert bei:											
		54°			56°			61°			65°		
	100	135	145	145	168	172	210	198	231	250	288	307	362
X-Mittelwert	100	142			183			226			319		

ten der Eiweisse studiert. Zu diesem Zwecke wurde das Pferdeserum oder das Hühnereiweiss von frisch gelegten Eiern (nach dem Filtrieren durch mehrere Marlischichten) in zwei Portionen geteilt. Eine dieser Portionen wurde nun 30 Minuten bei dieser oder jenen Temperatur denaturiert, während die andere Portion in nativem Zustande verblieb und als Kontrolle diente.

Auf Tabelle III sind die Ergebnisse der Methylenblauadsorbtion im nativen und denaturierten Serum wiedergegeben. Hier sehen wir die Hauptetappen unserer Zählungen, die uns zum X-Wert geführt hatten. Dieser X-Wert entspricht der Farbstoffmenge, welche vom denaturierten Eiweiss adsorbiert worden ist und wird in Prozenten der vom nativen Eiweiss gebundenen Quantitäten ausgedrückt. In den nachfolgenden Tabellen lassen wir die Nebenrechnungen aus und geben nur den X-Wert. Aus den Tabellen III u. IV folgt mit grosser Überzeugungskraft, dass die Wärmedenaturation der Serumeiweisse die Affinität zum Methylenblau steigert, wobei diese Verstärkung der Bindefähigkeit umso grösser ist, je höher die Denaturationstemperatur war. Wenn wir die Quantität des an das Eiweiss gebundenen Farbstoffes im nativen und denaturierten Serum vergleichen, so stellt es sich heraus, dass in ein und denselben Bedingungen das bei 68° denaturierte Serum eine um das 5-fache grössere Menge des Methylenblaues zu adsorbieren imstande ist. Es ist bemerkenswert, dass diese Steigerung der Adsorbtionsfähigkeit sich bereits auf sehr niedrigen Denaturationsstufen kundgibt. Schon nach halbstündligem Erwärmen bei 54°, wenn eine jegliche Spur von Trübung fehlt, erhalten wir eine reelle Färbbarkeitsverstärkung des Eiweisses (Tabelle IV).

Das denaturierte Serum zeigt eine erhöhte Affinität auch anderen basischen Farbstoffen gegenüber. Tabelle V und Tabelle VI illustrieren diese Erscheinung bei Tinktion mit Toluidinblau und Brillantkresylblau. Dasselbe Ergebnis erhalten wir bei Anwendung von Neutralrot.

Ähnliche Resultate ergaben die Versuche mit Hühnereiwess. Auf Tabelle VII finden wir die Daten für die Färbung mit Methylenblau und Toluidinblau eines Hühnereiweisses, welches während 30 Minuten bei 59° denaturiert worden war. Die Versuche haben gezeigt, dass in allen Fällen für beide Farbmittel eine Verstärkung der Färbbarkeit des denaturierten Eiweisses eintritt. *Die angeführten Resultate veranlassen uns zu behaupten, dass sowohl das Hühnereiweiss, als auch das Eiweiss des Blutserums nach der Wärmedenaturation ihre Affinität mit den basischen Farbstoffen verstärken.*

Der Denaturationsprozess ist nach Einwirkung verschiedenartiger Agentien nicht in allen Fällen identisch. Die erhaltenen Produkte können sich je nach der Art der angewandten Eiweissdenaturation ihrer Eigenschaften nach wesentlich voneinander unterscheiden. Deshalb ist es von grosser Wichtigkeit festzustellen ob die nach der Wärmedenaturierung auftretende Verstärkung der Adsorbtionsfähigkeit eine Besonderheit nur dieser Einwirkung ist oder ob sie auch bei anderen Denaturationsarten vorkommt. Zwecks Beantwortung

VOL. 24 1943

Tabelle V.

Wirkung der Hitzedenaturation auf die Toluidinblauadsorbtion (0,001 %) durch Eiweisse des Pferdeserums.			
Farbstoffadsorbtionswert X	Natives Serum	Denaturiert bei 66°	
	100	192	224
X-Mittelwert	100	208	

Tabelle VI.

Wirkung der Hitzedenaturation auf die Brilliantcresylblauadsorbtion (0,001 %) durch Eiweisse des Pferdeserums.			
Farbstoffadsorbtionswert X	Natives Serum	Denaturiert bei 63°	
	100	170	179
X-Mittelwert	100	174	

Tabelle VII.

Wirkung der Hitzedenaturation auf die Adsorbtion von basischen Farbstoffen durch Hühnereiweiss.							
Farbstoffadsorbtionswert X	Natives Eiweiss	Denaturiert bei 59°					
		Methylenblau 0,001 %			Toluidinblau 0,001 %		
	100	108	159	151	128	129	119
X-Mittelwert	100	139			125		

dieser Frage untersuchten wir die denaturierende Wirkung, welche Ultravioletttstrahlen, Äthylalkohol und Aceton auf die Adsorbtion des Blutserums ausführen.

Die Versuche mit den Ultraviolettstrahlen wurden folgendermassen gestellt: zur Bestrahlung diente die Lampe von BACH; die Spannung an den Lampenklemmen betrug 75 Volt, die Stromstärke 4,5 Ampère. Das Serum, welches als dünne Schicht den Boden der Petri-Schale bedeckte, war 50 Centimeter von der Lampe entfernt. Während der Bestrahlung stieg die Temperatur nicht über 26° C. Das native zum Vergleich dienende Serum wurde ebenfalls in eine Petri-Schale gegossen und gelangte — von der Einwirkung der Ultravioletttstrahlen geschützt — in die gleichen Wärmebedingungen. Nach verschiedener Bestrahlungsdauer wurde das bestrahlte und das native Serum in Kollodiumhülsen mit Methylenblau gefärbt. Tabelle VIII zeigt, dass 65-minutiger Bestrahlung keine bemerkenswerte Adsorbtionsverstärkung eintritt; doch nach länger andauernder Bestrahlung (2 Std. 30 Min.) wird die erhöhte Farbstoffadsorbtion deutlich wahrnehmbar. Somit ist auch bei ultravioletter Denaturation eine Verschiebung der Adsorbtionsfähigkeit im Eiweiss festzustellen. Sie sind den Vorgängen, die wir der Wärmedenaturation beobachten konnten, ähnlich.

VOL. 24 1943

Das Studium über die Wirkung der Wärme- und Strahlendenaturation auf das Tinktionsvermögen war durch den Umstand sehr erleichtert, dass zum Färbemoment sowohl das native als auch das denaturierte Eiweiss in streng gleichen Bedingungen sich befanden, da der denaturierende Faktor ohne Schwierigkeiten beseitigt werden konnte.

Bei der Alkohol- und Acetondenaturation mussten ebenfalls die gleichen Bedingungen eingehalten werden, da NASSONOV u. ALEXANDROV (1938) schon früher dargebracht hatten, dass Alkohol und Aceton selbst die Farbstoffadsorbtion stark beeinflussen. Um nun die Zusammensetzung der Lösungen des nativen und des denaturierten Eiweisses auszugleichen gebrauchten wir ein besonderes Verfahren. HARDY u. GARDINER (1910) haben gefunden, dass der Alkohol und das Aceton in der Kälte die Eiweisse des Blutserums vollkommen reversibel fällen. Bei Zimmertemperatur jedoch — unsere eignen Versuche haben es gezeigt — findet diese Reversität nur kurz nach der Einwirkung des Alkohols oder des Acetons statt. Wenn wir zu 5 ccm des Serums 5 ccm eines 96°-igen Äthylalkohols hinzugiessen, so entsteht ein ausgibiger Niederschlag, der sich aber leicht peptisieren lässt, wenn die Konzentration des Alkohols sofort bis unter die denaturierende Stufe herabgesetzt wird. Dieses kann durch Zusatz von 10 ccm der Ringer'schen Lösung oder des nativen Serums erzielt werden. Wird aber dieses Verdünnen 10 Minuten nach der Alkoholeinwirkung vorgenommen, so bleibt die Lösung des Niederschlages aus und die Eiweisskoagulation wird irreversibel. Die denaturierte Serumportion wird folgendermassen verfertigt: zu 10 ccm des Se-

Tabelle VIII.

Wirkung der Ultraviolettstrahlendenaturation auf die Methylenblauadsorbtion (0,001 %) durch Eiweisse des Pferdeserums.				
Farbstoffadsorbtionswert X	Natives Serum	Bestrahlungsdauer.		
		65 Min.	2 Std. 30 Min.	
	100	105	145	126
X-Mittelwert	100	105	135	

Tabelle IX.

Wirkung der Aethylalkoholdenaturation auf die Methylenblauadsorbtion (0,001 %) durch Eiweisse des Pferdeserums.				
Farbstoffadsorbtionswert X	Kontrollserum	Mit Aethylalkohol denaturiert		
	100	229	201	161
X-Mittelwert	100	197		

Tabelle X.

Wirkung der Acetondenaturation auf die Methylenblauadsorbtion (0,001 %) durch Eiweisse des Pferdeserums.			
Farbstoffadsorbtionswert X	Kontrollserum	Mit Aceton denaturiert	
	100	183	200
X-Mittelwert	100	191	

rums werden 5 ccm Äthylalkohol oder Aceton zugegossen. Sofort entsteht ein bedeutender Eiweissniederschlag der sich nun sehr bald als irreversibel denaturiert erweist. Nach c:a 30 Minuten wird diesem Gemische 5 ccm einer Ringer'schen Lösung zugegeben. Die Lösung bleibt trübe und das gefällte Eiweiss unpeptisiert, obgleich die endgültige Konzentration sowohl des Alkohols als auch des Acetons bei schwacher Verdünnung die Schwellendenaturation nicht erreicht. Die Serumportion, die zur Kontrolle diente, wurde aus denselben Lösungen zusammengestellt, doch in anderer Reihenfolge. Zu 5 ccm Alkohol oder Aceton gossen wir zuerst 5 ccm der Ringerlösung und erst darnach wurde der verdünnte Alkohol oder das Aceton mit 10 ccm Serum vermischt. In letzterem Falle reichte die Konzentration der denaturierenden Agentien zur Denaturation des Serums nicht aus und die Kontrollportion blieb — trotz der gleichen Zusammensetzung des Endproduktes — vollständig klar. Selbst wenn wir annehmen, dass auch in der Kontrollösung eine gewisse Denaturation des Eiweisses stattgefunden hatte, so muss jedenfalls der Grad dieser Denaturation bedeutend schwächer gewesen sein als in der ersten Lösung. Dieser Umstand berechtigte uns dazu die Kontrollösung als eine der nativen sehr nahestehenden Lösung zu betrachten und ihre Fähigkeit die Farbstoffe zu binden mit der Farbstoffaffinität der ersten zweifellos denaturierten Lösung zu vergleichen.

VOL. 24 1943

Nachdem der Ingradientenkomplex der denaturierten und der zur Kontrolle dienenden Eiweisslösung ausgegleichen war, wurden die Lösungen in Kollodiumhülsen gegossen und in 0,001 %-igem Methylenblau gefärbt. Die erhaltenen Resultate sind auf Tabelle IX und Tabelle X wiedergegeben. Wir sehen, dass das Eiweiss nach der Alkohol- oder Acetondenaturation im Vergleich mit der Kontrollösung das Methylenblau fast doppelt so stark bindet. *Somit werden alle untersuchten Denaturationsarten von einer erhöhten Adsorbtion der basischen Farbstoffe durch die Serum- oder Hühnereiweisse begleitet.*

Im Zusammenhange mit den Resultaten, welche wir mit basischen Farben erreicht hatten, war es interessant nachzuprüfen wie sich das Verhalten der denaturierten Eiweisse den sauren Farbstoffen gegenüber ändert. Die zu diesem Zwecke gestellten Versuche haben am Blutserum und am Hühnereiweiss ungleiche Resultate ergeben. Das bei 59° denaturierte Serum zeigte weder dem Cyanol, noch dem Trypanblau gegenüber eine Verstärkung der Adsorbtionsbindung. Wie es aus Tabelle XI zu ersehen ist, ist der Färbungsgrad des nativen Serums in beiden Fällen der Tinktion sehr ähnlich. Eine stärkere Denaturation, welche nach einer halbstündigen Erwärmung bei 62—60° eingetreten war (Tabelle XII), hatte gleichfalls keine bestimmte Veränderung der Adsorbtionsfähigkeit sauren Farbstoffen gegenüber gezeigt. Das Verhalten des Hühnereiweisses war jedoch ein anderes. Hier stieg die Fähigkeit des dena-

Tabelle XI.

Wirkung der Hitzedenaturation auf die Adsorbtion saurer Farbstoffe durch Eiweisse des Pferdeserums.			
Farbstoff-adsorbtionswert X	Natives Serum	Denaturiert bei 59°	
		Cyanol 0,002 %	Trypanblau 0,004 %
	100	97	101

Tabelle XII.

Wirkung der Hitzedenaturation auf die Cyanoladsorbtion (0,002 %) durch Eiweisse des Pferdeserums.						
Farbstoff-adsorbtionswert X	Natives Serum	Denaturiert bei:				
		62°		66°		
	100	96	92	98	120	90
X-Mittelwert	100	94		103		

Tabelle XIII.

Wirkung der Hitzedenaturation auf die Adsorbtion saurer Farbstoffe durch das Hühnereiweiss.			
Farbstoff-adsorbtionswert X	Natives Eiweiss	Denaturiert bei 59°	
		Cyanol 0,002 %	Trypanblau 0,001 %
	100	1.228	1.643

turierten Eiweisses das Cyanol zu binden um das 12-fache und das Trypanblau zu binden über das 16-fache (Tabelle XIII).

Wir haben oben gezeigt, dass die vitale Färbung des geschädigten Protoplasmas weder durch die Veränderung der Zellpermeabilität, noch durch die Erschöpfung ihrer Reduktionsfähigkeiten oder die Abspaltung der Nukleinsäure erklärt werden kann. Auch fehlen uns jegliche Gründe LEPESCHKIN beizustimmen, die für die Erklärung bestimmte Tinktionsfähigkeiten zuschreibt. Die dargelegten Tatsachen weisen uns vielmehr einen bedeutend einfacheren Weg für die Deutung dieser wichtigen, die Schädigung der Zelle stets begleitenden Erscheinung. In der Tat haben unsere Versuche mit den Blutserumeiweissen und dem Hühnereiweiss bewiesen, dass die erhöhte Farbstoffbindung nicht nur dem Protoplasma der lebendigen geschädigten Zelle eigen ist, sondern auch in Lösungen nativer Eiweis se in vitro bei Einwirkung verschiedenartiger alterierenden Agentien beobachtet wird. Dementsprechend wird der Parallelismus zwischen der kolloiden Reaktion der lebendigen Substanz und der nativen Eiweisslösung, welche sich in den sehr ähnlichen kolloidalen Umwandlungen unter dem Einfluss desselben äusseren Agentienassortiments kundgibt noch dadurch ergänzt, dass in beiden Systemen ausserordentlich ähnliche Veränderungen der Adsorbtionsfähigkeiten eintreten. Schwerlich kann dieser so weitgehende Parallelismus auf ein zufälliges Zusammenfallen der äusseren Charakteristik zweier, in Wirklichkeit verschiedenartiger Prozesse, zurückgeführt werden. Vielmehr liegt es nahe anzunehmen, dass die in der geschädigten Zelle sich abspielenden physikalisch-chemischen Vorgänge sich in den Veränderungen der nativen Eiweisse ihres Protoplasmas äussern. Dieselben müssen im Grunde den Veränderungen der nativen ausserhalb des Zellsystems befindlichen Eiweisslösung ähnlich sein. In Anbetracht dessen ist es notwendig an die Analyse der Prozesse heranzutreten, welche die Reaktion des nativen Eiweisses auf die verschiedenen Einwirkungen hervorrufen.

Die Veränderung des Kolloidzustandes der nativen Eiweisslösungen, welche sich in der Verminderung seiner Dispersität kundgibt, kann bereits fast mit allen, das Protoplasma lebendiger Zellen beschädigenden Agentien bewirkt werden. Diese Kolloidveränderungen sind mit hilfe folgender Einwirkungen erhalten worden: hohe Temperatur, Kälte (VICKERY 1926; FINN 1932), Strahlenwirkung: sichtbare Strahlen (JOUNG 1922; CLARK 1922), Ultraviolette Strahlen (BOVIE 1913 a, b; MOND 1922, 1923; CLARK 1925, 1935, 1936; SPIEGEL-ADOLF 1927, 1928, 1934 u. and.), Röntgenstrahlen (WELS 1923, 1924, 1937; WELS u. THIELE 1925; NAKASHIMA 1926; RAJEWSKY 1930), β-Strahlen des Radiums (FERNAU u. PAULI 1915, 1922; FERNAU u. SPIEGEL-ADOLF 1929), Ultra-Lautwellen (SCHMIDT, OLSON u. JOHNSON 1928; WU and LIN 1931), hörbare Wellen (FLOSDORF u. CHAMBERS 1935; CHAMBERS and FLOSDORF 1936), Adsorbtion an verschiedenen Grenzflächen (RAMSDEN 1894, 1903, 1904; SPIEGEL-ADOLF 1932), mechanische Einwirkung

VOL. 24 1943

(HERZFELD u. KLINGER 1917; WIECHOWSKI 1917), hoher atmosphärischer Druck (BRIDGMAN 1914; BRIDGMAN u. CONAUT 1929; PORSCHEZ 1925), genügende Konzentrationen vieler Salze und gleichfalls herabgesetzte Salzkonzentrationen (Globulinfällung); Säuren, Narkotika (BATTELLI u. STERN 1913; LEPESCHKIN 1923; WU 1927 u. and.), Alkaloide (SCHARUKE 1922; FREY 1922), Harnstoff (HOPKINS 1930), sogenannte hydrotope Stoffe (Salicylaten, Benzoaten, Rhodanaten, Formamid u. a.) (PAULI u. WEISS 1931; AUSON and MIRSKY 1929), Wasserhyperoxyd (SCHULZ 1898; FERNAU u. SPIEGEL-ADOLF 1929).

Mit den erwähnten Tatsachen ist die grosse Mannigfaltigkeit der Faktoren, welche die Stabilität des nativen Sols herabsetzen und die Aggregation seiner Teilchen hervorrufen können, noch nicht erschöpft. Das Dargelegte genügt aber vollkommen um sich von der ausserordentlichen Labilität dieses Kolloidsystems und von ihrer Fähigkeit die mannigfaltigsten Einwirkungen in ähnlicher Weise zu beantworten zu überzeugen.

Die Dispersionsverminderung mit welcher das native Eiweiss auf die verschidenartigsten Faktoren reagiert, kann nur in wenigen Fällen auf die Einwirkung der gegebenen Agentien auf die Eiweissladung, die die elektrische Konstante der Lösung oder auf die Dehydration der Solvathülle der Micelle zurückgeführt werden. Den meisten hier genannten Kolloidveränderungen liegen tiefere Umwandlungen der Eiweissmoleküle selbst zu Grunde die ihre Hydrophilie herabsetzen. Die Leichtigkeit, mit welcher der native Zustand der Eiweissmoleküle gestört wird, bedingt die Eigenartigkeit der kolloidalen Reaktionen des nativen Eiweisses und grenzt sie in vielen Fällen von der Reaktion aller übrigen Kolloidenlösungen scharf ab. So z. B. sind uns bis jetzt keine Emulsoide oder Suspensoide bekannt, die — gleich dem nativen Eiweisse — bei 60—70° C. koagulierten. Wie es von einer Reihe von Forschern festgestellt worden ist, rufen die Röntgen- und β-Strahlen eine Koagulation und Herabsetzung der Stabilität nur in positiv geladenen eiweissfreien Kolloiden hervor; negativ-geladene Kolloide werden dabei stabiler und stärker dispergiert (HENRI et MAYER 1904; GROWTHER and FAIRBROTHER 1927). Das Verhalten des nativen Eiweisses denselben Agentien gegenüber ist nun grundverschieden. WELS u. TIELE 1925 haben eine Herabsetzung der Hydrophile des Blutalbumins und Globulins nach Röntgenbestrahlung zu beiden Seiten des isoelektrischen Punktes erhalten. FERNAU u. PAULI 1925; FERNAU u. SPIEGEL-ADOLF 1929 haben gleichfalls gezeigt, dass im Gegensatz zu den anorganischen Kolloiden die durch die β-Strahlen hervorgerufene Veränderung der Kolloideigenschaften des Serumalbumins von der Ladung der Eiweissteilchen unabhängig ist; dementsprechend kann sie durch die Bestrahlung sowohl saurer als auch basischer Eiweisslösungen erzielt werden.

Die Notwendigkeit ausser den kolloidalen Veränderungen auch noch die molekularen Umbildungen bei der Einwirkung auf das native Eiweiss im Auge zu

behalten hat bereits HARDY im Jahre 1899 bewiesen. Später hat eine Reihe von Forschern die Möglichkeit einer Absonderung dieser beiden Prozesse vielfach geschildert. Mittels einer Veränderung der Wasserstoffionenkonzentration oder der Salzzusammensetzung der Eiweisslösung ist es möglich beim Erwärmen jegliche wahrnehmbare Kolloidumwandlungen auszuschliessen; trotzdem ist das Eiweiss vollständig verändert und nun unfähig nach dem Entfernen der Elektrolyten in der Lösung zu verbleiben. Nicht weniger überzeugend ist dieses in Bezug auf die Wirkung der Strahlenenergie (BOVI 1913; FERNAU u. SPIEGEL-ADOLF 1929; CLARK 1935) des Alkohols (WU 1927) und anderer Agentien am Eiweiss gezeigt worden. Schliesslich liefern die röntgenologischen Untersuchungen ASTBURYS und seiner Mitarbeiter (ASTBURY 1936, 1938; ASTBURY, DICKINSON and BAILEY 1935; ASTBURY and LOMAX 1935) unwiderrufliche Beweise für die Gegenwart von Molekularveränderungen während der Eiweissdenaturation. Alles Obenerwähnte widerspricht BANCROFTS u. RUTZLERS Angaben (1931), laut welchen die Denaturation auf ein einfaches Kolloid-chemisches Phänomen zurückzuführen ist.

VOL. 24 1943

Wenn wir ein Urteil davon haben wollen, wie weit die in vitro beobachteten Veränderungen der Eiweisslösung den Umwandlungen, welche im Protoplasma der lebendigen Zelle sich abspielen, verwandt sind, müssen wir die Frage über die Reversibilität dieser Veränderungen besprechen. Dabei ist es nötig zwei Fälle im Auge zu behalten erstens, wenn es für die Rückkehr des Stoffes in seinen normalen Zustand genügt, die Ursache dieser Umwandlungen auszuschalten und zweitens, wenn ausser dieser Massregel noch eine andere hinzugesetzt werden muss, nämlich eine Nachbehandlung des veränderten Produkts. Im ersten Falle sprechen wir von einer homodromen, im zweiten — von einer heterodromen Reversibilität. Wenn schliesslich die Rückkehr zum Ausgangszustand überhaupt nicht möglich ist, so wird die entsprechende Veränderung als endgültig irreversibel bezeichnet.

Beim Studium der Umwandlungen des nativen Eiweisses infolge verschiedenartiger Einwirkungen stossen wir auf alle drei Erscheinungskathegorien. Die homodrom irreversiblen Veränderungen führen gewöhnlich den Namen „Denaturation" (PAULI u. WALKO 1933). Es ist jedoch nicht immer möglich die Eiweissdenaturation von anderen Fällen — wenn bloss eine homodrom reversible Veränderung der kolloidalen Eigenschaften stattfindet — zu unterscheiden. Tatsächlich kann im gleichen Eiweisse ein und derselbe Faktor in Abhängigkeit von seiner Intensität, der Wirkungsdauer und den Wirkungsbedingungen reversible und irreversible Umwandlungen hervorrufen. Die leicht reversible Fällung der Muskelglobuline, welche durch die Herabsetzung oder Verstärkung der Salzkonzentrationen bedingt wird, führt bei längerem Verbleiben des Eiweisses im Niederschlage zum irreversiblen Verluste seiner Auflösungsfähigkeit (EDSALL 1930). Das mittels Alkohol oder Aceton gefällte Blutserumeiweiss geht in der Kälte (bei 0° C.) nach dem Entfernen die-

ser Agentien mit Leichtigkeit in die Lösung über. Unsere Beobachtungen zeigen, dass bei Zimmertemperatur eine vollständige homodrome Reversibilität der Koagulation nur während der ersten Minuten möglich ist; bei längerer Alkoholwirkung bleibt der Niederschlag ungelöst. PAULI u. WEISS (1931) haben eine Herabsetzung der homodromen Reversibilität des gefällten Eiweisses sowohl bei andauernder Alkoholwirkung als auch bei erhöhter Alkoholkonzentration beschrieben. Schliesslich erwähnen wir noch die Befunde von MICHAELIS u. RONA P. (1910), laut welchen die kurzandauernde Wärmekoagulation des Serumalbumins ein Produkt ergab, welches fähig war nach der Behandlung mit Salzsäure zu seinem nativen Zustand zurückzukehren. Bei länger andauerndem Erwärmen erhielten diese Forscher ein Koagulat, welches selbst zur heterodromen Reversibilität unfähig war.

Wenn wir den Begriff „Denaturation" nur in den Fällen eines homodrom irreversiblen Verlustes der Hydrophilie des nativen Eiweisses gebrauchen wollen, so müssen wir uns die Relativität einer solchen Absonderung vergegenwärtigen und nicht vergessen, dass es Zustände gibt, die einen Übergang von der typischen Denaturation zu leicht reversiblen Veränderungen bilden.

Bei den Versuchen, welche im experimentellen Teil der vorliegenden Arbeit dargelegt waren, hatten wir es mit der echten durch Wärmewirkung, Alkohol, Aceton und Ultraviolettstrahlen hervorgerufenen Eiweissdenaturation zu tun. Hinsichtlich aller dieser Agentien ist es bekannt, dass unter entsprechenden Bedingungen die denaturierende Wirkung im Zusammenhange mit den Kolloidumbildungen auch zu homodrom irreversiblen Veränderungen der Eiweissmoleküle führen. Deshalb ist es von Interesse zu ermitteln ob die beobachtete Intensität der Farbstoffbindung als Folge einer Aggregation der Eiweisslösungspartikel auftritt oder ob sie vom molekulären Umbau, der diesem Kolloideffekt zu Grunde liegt, abhängt.

Die unten geschilderten Tatsachen zeigen, dass die Veränderung des kolloidalen Eiweisszustandes für die erhöhte Farbstoffaffinität des Eiweisses nicht verantwortlich gemacht werden kann.

In den entsprechenden Versuchen wurde der Koagulationsgrad nach der Intensität des Tindalstrahls im Pulfrich-Nephelometer festgestellt und in relativen Trübungseinheiten an der Scala dieses Instruments abgelesen. Beim Experimentieren konnten wir eine interessante Erscheinung beobachten. Wenn wir das durch Erwärmen denaturierte Blutserum mit der Ringerlösung 2, 4, 8-mal verdünnen, wird am Anfang die Trübung der Lösung resp. der Koagulationsgrad des Eiweisses trotz der geschwächten Eiweisskonzentration und des unveränderten Salzgehaltes nicht herabgesetzt, sondern stark erhöht. Auf Abb. I sind zwei Kurven dargestellt, die den Verlauf der Trübungsveränderungen des denaturierten Serums in Abhängigkeit vom Lösungsgrad wiedergeben. Im Serum, welches bei 64° während einer halben Stunde denaturiert war, stieg die Trübung der vierfach verdünnten Eiweisskonzentration

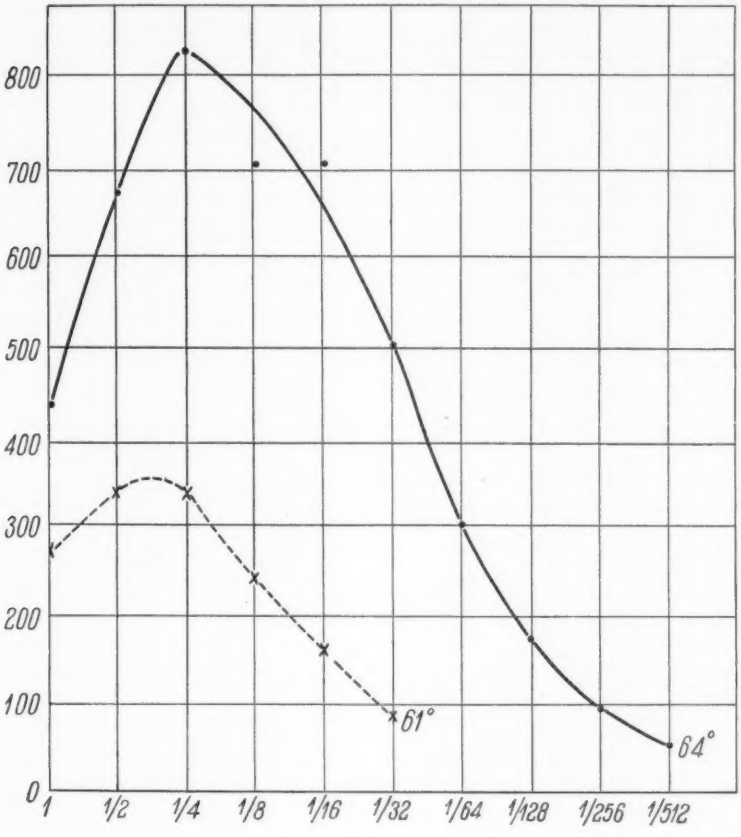


Abb. 1.

fast um das Doppelte. Bei weiterer Abschwächung fiel die Trübung, doch war der weitere Verlauf dieser Abnahme fast bis zum Ende der Eiweisskonzentrationsunterdrückungskurve nicht proportionell. Dasselbe Resultat, wenn auch weniger deutlich, zeigt auch die zweite Kurve eines schwächer denaturierten Serums (61° C.).[1] Die Ursache dieser Erscheinung ist uns nicht klar; wir können jedoch annehmen, dass beim Verdünnen der Lösung bessere Bedingungen für die Realisierung der Kolloidfolgen der durch Hitzedenaturation hervorgerufen Molekularveränderungen geschaffen werden. Dieses Phänomen ist für die Lösung der obenerwähnten Frage über die Wechselbeziehungen zwischen Adsorbtions- und Kolloidveränderungen benutzt worden.

[1] Unsere Beobachtungen stehen in gutem Einklange mit den Befunden von KIYOTAKI (1922), der gezeigt hat, dass bei der Verdünnung eines normalen Serums die Trübung der Eiweisskonzentrationherabsetzung nachsteht. Wie bekannt, ist die Trübung in anderen Fällen der Stoffkonzentration in der Lösung in weiten Grenzen direkt-proportioneli.

VOL. 24 1943

Das bei 64° denaturierte unverdünnte oder mit der Ringerlösung 3-fach verdünnte Serum wurde mit Methylenblau in Kollodiumhülsen gefärbt. Die Trübung des intakten Serums, welche 588 Einheiten betragen hatte, stieg nach der Verdünnung bis 952 (Die Trübung des nativen, nicht verdünnten Serums entsprach 90 Einheiten). Somit war die Koagulation bedeutend stärker in der nach der Denaturation verdünnten Lösung. Dementgegen band in dieser Lösung jede Eiweisseinheit den Farbstoff I, 35-Mal weniger als die Eiweisseinheit des schwächer koagulierten Serums. Auf Grund dieses Versuches muss angenommen werden, dass die Koagulation als solche die Farbstoffaffinität nur herabzusetzen vermag.

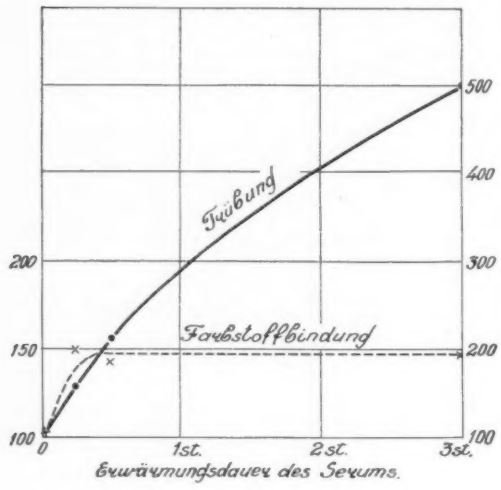


Abb. 2.

Die Nichtübereinstimmung der Aggregationsverstärkung mit dem Aufstieg der Farbstoffaffinität findet ihren Ausdruck auch in der verschiedenen Abhängigkeit dieser beiden Erscheinungen von der Dauer der Wärmedenaturation. Die vorläufigen Versuche haben gezeigt, dass der Wuchs der Adsorbtionsfähigkeit nur zu Beginn des Erwärmens beobachtet wird, wonach eine weitere Adsorbtionsverstärkung — trotz der fortschreitenden Trübung — nicht stattfindet. Die Trübung jedoch wird mit der Erwärmungsdauer immer bedeutender.

Das Resultat eines solchen Versuches ist auf Abb. 2 wiedergegeben. Wir sehen, dass die Methylenblauaffinität infolge der Erwärmung des Serums bei 57° schon nach 15 Minuten ihr Maximum erreicht. Die fortgesetzte Denaturation bei dieser Temperatur kann die Farbstoffaffinität des Eiweisses nicht mehr erhöhen, obgleich die Koagulation fortschreitet. Das von uns festgestellte Bindungsnivau, welches bei der gegebenen Temperatur schon während der ersten 15 Minuten erreicht wird, steht in gutem Einklange mit FISCHERS (1935) Beobachtungen, worüber unten noch die Rede sein wird.

Die erhaltenen Resultate zeigen, dass die Veränderung der Adsorbtionsfähigkeit bei der Eiweissdenaturation die Folge der hier eingetretenen Molekularveränderungen ist und nicht als Resultat einfacher Kolloidumbildungen des Eiweissystems betrachtet werden darf.

Bei der Besprechung dessen, welche Molekularumbauungen für die Verstärkung der Farbstoffaffinität während der Eiweissdenaturation verantwort-

lich gemacht werden müssen, ist es nötig die Natur der Kräfte, welche diese Bindungen hervorrufen, zu berücksichtigen. In seiner zusammenfassenden Arbeit über die Theorie der histologischen Färbung spricht MICHAELIS (1920) eine den alten Ansichten von EHRLICH und HEIDENHAIN entsprechende Ansicht aus und gibt die Möglichkeit einer chemischen Reaktion zwischen dem Farbstoff und den denaturierten Eiweissen des histologischen Präparates zu. Das Eiweiss erzeugt salzartige Verbindungen sowohl mit sauren als auch mit basischen Farbstoffen aufkosten seiner Amin- und Carboxylgruppen. Diese Auffassung hat ihre Bestätigung in der Arbeit von UMETSU (1923) erhalten. Der Autor beweist an einer Reihe von Eiweissen die Verschiebung des isoelektrischen Punktes in die saure Seite bei Einwirkung saurer Farbstoffe und die Verschiebung in die alkalische Seite bei der Bindung basischer Farbstoffe.

Sehr wesentliche Argumente finden wir auch in den Arbeiten von STEARN and STEARN (1929) und STEARN (1930). Hier wird die Konzentrationserhöhung der Wasserstoffionen in den Lösungen bei Eiweissbindungen saurer Farbstoffe und die Konzentrationsabnahme bei Anwendung basischer Farbstoffe geschildert. Wenn wir uns die obengenannten Tatsachen und die von VALKO (1937) in seiner zusammenfassenden Arbeit dargelegten Beobachtungen vergegenwärtigen, so sind wir wohl gezwungen zuzugeben, dass bei unseren Eiweissen die Farbstoffbindung von den Hauptvalenzen der Carboxyl- und Amingruppen geschaffen wird, — zum mindesten in den Fällen, wenn die Tinktion mittels saurer oder alkalischer Farbstoffe mit schwach ausgedrückten substantiven Eigenschaften erfolgt.

Sollte diese Annahme richtig sein, so ist zu erwarten, dass die Denaturation des Eiweisses, welche die Farbstoffbindung steigert, zu einer Erhöhung der Affinität auch der einer Reihe anderer Stoffe führt. Leider ist die Veränderung der Bindungsfähigkeit der denaturierten Eiweisse fast garnicht studiert. Eine Ausnahme bilden nur die Bindungen starker Säuren und Basen. Deshalb sind *Fischers* Beobachtungen von grossem Interesse. Er hat die Wirkung der Hitzedenaturation auf die Bindung des Heparins an Hühnerblutplasma und Eialbumin studiert. Das Heparin, dessen Struckturformel bis jetzt nicht sicher bestimmt ist, besitzt die Bruttoformel $C_{18}H_{32}O_{17}6H_2O$ und enthält eine Säuregruppe. Es unterliegt wohl keinem Zweifel, dass die Bindung des Heparins — dieser organischen Säure — durch die Amingruppen des Eiweisses erfolgt. Die von *Fischer* festgestellte Bindungssteigerung des Heparins steht in gutem Einklange mit der von uns beobachteten Affinität des denaturierten Hühnereiwesses zu sauren Farbstoffen. Zu Gunsten der Annahme, dass der erhöhten Affinität des denaturierten Eiweisses zum Heparin und zu den von uns untersuchten Farbstoffen ein und dieselben molekulären Ursachen zugrunde liegen, spricht die vollkommene Übereinstimmung in der Wirkung der Denaturationsdauer auf die Verstärkung dieser Bindung. Abb. 3 zeigt eine Kurve aus *Fischers* Arbeit. Wir sehen die Abhängigkeit der He-

VOL. 24 1943

parinbindung sowohl von der Temperatur als auch von der Dauer des Verbleibens des Eiweisses in dieser Temperaturbedingung. In Einklang mit den Farbstoffversuchen (Tabelle III u. IV) wird das Heparin umsomehr vom denaturierten Eiweiss gebunden, je höher die Denaturationstemperatur gewesen ist (bis 70° C.). Anderseits war laut unseren Beobachtungen (Abb. 2) der entsprechende Höhepunkt der Heparinbindung schon 10 Minuten nach der Übertragung des Eiweisses in die nötige Temperatur erreicht und konnte nur durch Temperaturerhöhung (58°, 70°) verändert werden — nicht aber durch eine Verlängerung der Wirkungsdauer der gegebenen Temperatur. Die von FISCHER beobachtete Abnahme der Heparinbindung bei Einwirkung höherer Temperaturen (80°—100°) wird vom Verfasser durch die starke Abnahme der Eiweissdispersion gedeutet. Diese Erklärung entspricht vollkommen unserer Ergebnissen über die Wirkung der Verdünnung des denaturierten Serums auf seine Färbbarkeit.

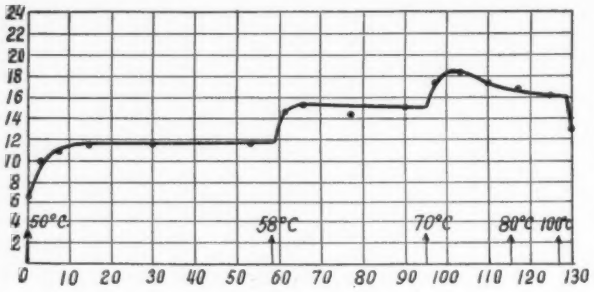


Abb. 3.

Die dargelegten Tatsachen zwingen uns anzunehmen, dass bei der Eiweissdenaturation entweder eine Vergrösserung der Zahl der freien H^+ resp. OH^--gruppen oder eine Verstärkung ihrer Ionisierung stattfindet.

Wir besitzen in der Literatur zahlreiche Angaben über die Verschiebung des isoelektrischen Punktes der in verschiedener Weise denaturierten Eiweisse (MICHAELIS u. DAVIDSOHN 1911; PEDERSEN 1931; WU u. YANG 1931; FAY and HENDRIX 1931; MAINZER 1932 u. and.). Diese Beobachtungen sprechen zweifelsohne zugunsten der durch Denaturation hervorgerufenen Veränderung der sauer-alkalischen Eigenschaften des Eiweisses, doch geben sie keine ausreichende Charakteristik dieser Umwandlungen. So beweist die Verschiebung des isoelektrischen Punktes im alkalischen Sinne nur eine gewisse Veränderung in dem Wechselverhältnisse zwischen den Amin- und Carboxylgruppen; wir können aber noch nicht bestimmen, ob die Verschiebung auf Kosten einer Verstärkung der Amingruppen oder infolge der Schwächung der entgegengesetzt geladenen Gruppen entstanden ist. Ebenso sind wir nicht berechtigt beim Fehlen einer Verschiebung des isoelektrischen Punktes die Möglichkeit gleichzeitiger äquivalenter Veränderungen in der Zahl der Amin- und Carboxylgruppen oder des Ionisierungsgrades zu leugnen.

Ausreichendere Vorstellungen von der Wirkung der Denaturation auf die Amingruppen geben uns die Beobachtungen über die Veränderung der Wasserstoffionenkonzentration bei der Denaturation und der Vergleich der Titrationskurven des nativen und denaturierten Eiweisses. Die diesbezügliche Literatur ist recht ausgibig, doch enthält sie wesentliche Widersprüche nicht nur in der Erklärungsweise der beobachteten Vorgänge, sondern auch in der Feststellung des Charakters der eingetretenen Veränderungen. MICHAELIS u. RONA, welche bereits im Jahre 1909 die Verminderung der Acidität infolge der Hitzedenaturation des Blutserums beschrieben hatten, erklären diese Erscheinung durch die Ausscheidung von Kohlensäure. Das Fehlerhafte dieser Deutung haben SÖRENSEN and JÜRGENSEN (1911) und QUAGLIARELLO (1912) bewiesen. SÖRENSEN und JÜRGENSEN machen die herabgesetzte Konzentration des freien, aus der Lösung gefällten Eiweisses für die Verminderung der Acidität verantwortlich. Die Arbeiten von CHICK u. MARTIN (1911, 1913) stellen fest, dass bei der Wärmekoagulation nicht nur eine Herabsetzung der Acidität in sauren Lösungen, sondern auch eine Abschwächung der Alkalität in alkalischen Eiweisslösungen beobachtet wird.

VOL. 24 1943

Im Gegensatz zu SÖRENSEN und JÜRGENSEN behaupten CHICK und MARTIN dass bei der Hitzekoagulation nicht das freie Eiweiss ausfällt, sondern das nicht hydrolysierte Eiweisssalz der Base oder Säure. Dieser Umstand führt zu einer Verschiebung der Wechselbeziehung zwischen dem hydrolysierten und nicht hydrolysierten in der Lösung zurückbleibenden Eiweisssalze. Das gestörte Gleichgewicht wird durch die entsprechende Mengenverminderung des hydrolysierten Produktes infolge der Bindung der freien Säure oder der freien Base durch das Eiweiss wieder hergestellt. Somit geben diese Autoren eine Deutung der erhöhten Säure- und Alkalibindung während der Denaturation ohne die Notwendigkeit einer Bildung neuer Amin-Carborcylgruppen oder einer Verstärkung ihrer Ionisierung anzuerkennen.

Die späteren Arbeiten haben viele neue Widersprüche geschaffen. So vergleichen LEWIS (1927), BOOTH (1930) und LOUGHLIN (1933) die für das native und denaturierte Eiweiss erhaltenen $H^+ - OH^-$ Titrationskurven und finden keinerlei Einwirkung der Denaturation auf die Fähigkeit des Eiweisses Säuren oder Basen zu binden. HENDRIX u. WILSON (1928) denaturieren das Hühneralbumin bei isoelektrischer Reaktion mittels Erwärmen im Autoklave und stellen durch Titration eine Herabsetzung der Tätigkeit des denaturierten Eiweisses Säuren und Basen zu binden fest. Dieselben Resultate ergab die in der Kälte vorgenommene Säuredenaturation des Fibrinogens (FAY u. HENDRIX (1931)). Später haben HENDRIX u. WARTON (1934) das Hühnereialbumin bei verschiedenen pH erwärmt und BOOTH's Beobachtungen über das Ausbleiben von Veränderungen in den Puffereigenschaften bei neutraler Denaturation bestätigt; auch konnte eine Verschiebung im Albumin, welches bei pH3 denaturiert war, nicht festgestellt werden. Im Einklange mit den Be-

funden von HENDRIX u. WILSON finden diese Forscher wieder eine Herabsetzung der Bindungsfähigkeit desselben Eiweisses, welches jedoch in seinem isoelektrischen Punkte denaturiert war.

BOOTH u. LOUGHLIN weisen mit Recht darauf hin, dass die von HENDRIX u. WILSON angewandte Methode eine so grobe ist, dass die erhaltenen Resultate nicht von der Denaturation selbst, sondern von den nachfolgenden tiefer eingreifenden Veränderungen abhängig sein können. Dennoch gebrauchen HENDRIX u. WARTON in ihrer Arbeit vom Jahre 1934 dieselbe Technik.

In krassem Widerspruch mit diesen Befunden stehen die Versuche BARKERS (1933), welcher die alten Ergebnisse von SÖRENSEN u. JÜRGENSEN, QUAGLIARELLO, CHICK u. MARTIN (1911) bestätigt und beweist, dass die Hitzedenaturation des Hühneralbumins bei saurer Reaktion die Herabsetzung der Wasserstoffionenkonzentration, bei alkalischer Reaktion die Herabsetzung der hydroxylen Ionenkonzentration hervorruft.

Auf Grund der oben geschilderten Tatsachen wäre es sehr schwer zu irgendeinem positiven Ergebnis hinsichtlich des Einflusses der Denaturation auf den Zustand der aminosauren Eiweissmolekülgruppen zu gelangen. Die Untersuchungen von WU u. CHEN (1929) erleichtern jedoch die Lösung dieser Frage und erklären uns infolge welcher Umstände die verschiedenen Autoren zu entgegengesetzten Resultaten haben kommen können. In ihrer ausserordentlich sorgfältigen Arbeit führen WU u. CHEN eine scharfe Grenze zwischen der Eiweissdenaturation und den Koagulationserscheinungen. Bei langsamen Erwärmen des nativen Eiweisses in schwach alkalischen oder sauren Lösungen erhielten diese Forscher eine Eiweissdenaturation, die von einer Eiweissfällung nicht begleitet war. Das in dieser Weise denaturierte Eiweiss bindet bei nachfolgender Übertragung in ein saures oder alkalisches Mittel bedeutend grössere Mengen der Wasserstoff — oder Hydroxylionen als das native Eiweiss. Diese erhöhte Bindefähigkeit ist umso stärker je weiter das pH, bei welchem die Denaturation vorgenommen war, vom isoelektrischen Punkte entfernt war. Dieselbe Zunahme der Bindefähigkeit den H und OH-Ionen gegenüber hat WU (1927) auch bei Alkoholdenaturation gefunden. Die Ausmessungen von WU u. CHEN zeigen eine Konzentrationsabnahme der freien Säure oder Base während der Denaturation ohne Eiweissfällung und zwingen uns SÖRENSENS u. JÜRGENSENS, CHICKS u. MARTINS Deutung abzulehnen. Die chinesischen Forscher behaupten, dass die von ihnen erhaltenen Tatsachen die Denaturation des Eiweisses auf die schwache Hydrolyse desselben zurückführen lassen. Anderseits haben WU u. CHEN festgestellt, dass die beim Erwärmen der isoelektrischen Lösung am stärksten ausgedrückte Eiweisskoagulation an und für sich nur zu einer Abnahme der Fähigkeit des Eiweisses die Wasserstoff- und Hydroxylionen zu speichern führt. Infolgedessen erhalten wir bei der Eiweisserwärung unter verschiedenen pH ungleiche Resultate — in Abhängigkeit vom Vorherrschen dieser

oder jener entgegengesetzten Tendenz. Wu u. Chen's Hinweis auf die durch Koagulationwirkung herabgesetzte Bindefähigkeit des Eiweisses steht in gutem Einklange sowohl mit unseren Veränderungsversuchen des denaturierten Serums, als auch mit Fischers Versuchen, laut welchen die Heparinbindung bei 80° überschreitenden Temperaturen unterdrückt wird. *Die Beobachtungen an der Wirkung der Denaturation auf die Säure — und Basenbindung durch die Eiweisse gestatten uns also die festgestellte Färbbarkeitssteigerung des denaturierten Eiweisses durch die erhöhte Aktivität der freien* H^+- *und* OH^--*gruppen oder durch die Zunahme ihrer Zahl zu erklären.*

Die Untersuchungen der Farbstoffbindungen während der Denaturation können wahrscheinlich solche Veränderungen der sauer — basischen Eigenschaften des Eiweisses klarlegen, welche in den Versuchen mit starken Säuren und Basen nicht in Erscheinung treten. In den besonders interessanten Arbeiten von Porai-Koschitz und seinen Mitarbeitern (1937 a, b, c) über die Färbung von Wolle und Seide mit sauren Farbstoffen finden wir Hinweise auf die Richtigkeit unserer Annahme. Diese Forscher haben gezeigt, dass die Farbstoffe sich mit den genannten Eiweisssubstraten beim Einhalten der stechiometrischen Verhältnisse chemisch vereinigen. Bei Färbung mit verschiedenen Farbstoffen können jedoch 2 Sättigungsgrenzen erreicht werden. Z. B. bindet 1 Gramm Wolle bei Sättigung mit einigen Farbstoffen 0,80—0,83 mg/äquiv. Dieser Wert entspricht ebenso der Salzsäuresättigung der Wolle und zeigt somit, dass in beiden Fällen die Bindung durch ein und dieselben Amin-Eiweissgruppen erfolgt. Ausserdem gibt es aber Farbstoffe, denen eine geringere Sättigungsgrenze — 0,60—0,65 Mq/äquiv auf je 1 Gramm Wolle — eigen ist. Porai-Koschitz nimmt an, dass in der Wolle zwei verschieden aktive Kathegorien der Eiweissgruppen vorhanden sind. Starke Säuren bilden Salze mit beiden Gruppen, wogegen schwache Säuren sich nur mit mehr aktiven Amingruppen verbinden. Wenn im Eiweisse infolge irgend welcher Einwirkungen eine Veränderung der Wechselverhältnisse zwischen den schwachen und starken Amingruppen — ohne jedoch ihre Gesamtzahl zu beeinflussen — eintritt, muss diese Verschiebung in der Bindung der Farbstoffe, denen eine niedrige Sättigungsgrenze eigen ist, ihren Ausdruck finden; bei Anwendung starker Säuren und Farben der ersten Kathegorie müsste sie nicht in Erscheinung treten. Somit können wir nicht mit Bestimmtheit behaupten, dass in allen Denaturationsfällen die erhöhte Bindungsfähigkeit des Eiweisses mittels beliebiger Farbstoffe darstellbar sei.

Der tiefe Parallelismus der Erscheinungen im beschädigten Zellprotoplasma einerseits und der Veränderungen, die sich in den Eiweisslösungen während ihrer Denaturierung abspielen, anderseits — zwingt uns anzunehmen, dass die Beschädigung der lebendigen Zelle von Veränderungen der in der lebendigen Substanz vorhandenen Eiweisse begleitet wird, welche den in vitro beobachteten Umwandlungen sehr ähnlich sind. Von diesem Standpunkte aus muss zu-

VOL. 24 1943

gegeben werden, dass die bei der Denaturation der Eiweisslösungen festgestellten Gesetzmässigkeiten im Allgemeinen auch bei der in der geschädigten Zelle sich abspielenden Vorgänge angewandt werden können. *Deshalb sind wir geneigt das Auftreten diffuser Protoplasma- und Kernfärbungen der alterierten Zellen durch die erhöhte Aktivität der* H^+ *und* OH^- *Gruppen der sich denaturierenden Eiweissmoleküle der lebendigen Substanz zu erklären.*

Eine weitere Folgerung der Denaturation der Zelleiweissmoleküle bildet die gewöhnlich hier eintretende Dispersionsverminderung der Kern- und Plasmakolloide und die Veränderung ihrer Viscosität.

Wie erwähnt, werden die verschiedenartigen Beschädigungen von der Verschiebung der intracellularen Reaktion in die saure Seite sehr oft begleitet. Diese Erscheinung ist von zahlreichen Forschern bei Einwirkung mannigfaltiger Agentien beobachtet worden Erstickung (NASSONOV 1930, 1932; ALEXANDROV 1932; TRIFONOVA 1935), hohe Temperatur (BRAUN u. IWANOV 1933; AISENBERG 1934; MESCHTSCHERSKAJA 1935; PRIWOLNJEW 1935), Einfrierung (RUNNSTRÖM 1935), mechanische Beschädigung (PARAT 1928), Narkotika (MAKAROV 1936), H_2S (SUTULOV 1937), Hypotonis (RUNNSTRÖM 1935; KAMNEV 1934) u. s. w.

Der Grund für die erhöhte Säurereaktion der beschädigten Zelle ist naturgemäss im Auftreten entsprechender Verschiebungen im intracellulären Stoffwechsel zu suchen. Besonders drängt sich dieser Gedanke bei den Erstickungsversuchen auf. Hier muss tatsächlich die Möglichkeit einer Speicherung saurer unoxydierten Produkte — in erster Linie die Konzentration von Milchsäure zugegeben werden. Auch bei der Einwirkung anderer Faktoren ist es leicht die Gegenwart von Verschiebungen in den Wechselverhältnissen zwischen den Oxydations- und Spaltungsprozessen anzunehmen. Diesen Gedanken unterstützen besonders die Untersuchungen von WARBURG (1926), welcher die erhöhte aerobe Gärung als erstes chemisches Anzeichen der Zellbeschädigung betrachtet.

Die zu unserer Verfügung stehenden Kenntnisse inbetreff der Reaktion des Mittels bei der Eiweissdenaturation gestatten uns neben der Stoffwechselstörung auch noch einen anderen Mechanismus für diese Erscheinung verantwortlich zu machen. Die Untersuchungen von CHICK u. MARTIN (1913), WU u. CHEN (1929), BARKER (1933) u. and. haben gezeigt, dass bei der Eiweissdenaturation in schwach-alkalischen Lösungen die Verschiebung der Reaktion in die saure Seite auf Kosten der Bindungsverstärkung der Hydroxylionen durch das Eiweiss erfolgt. Sollte es richtig sein, dass bei der Zellbeschädigung eine Denaturation der Protoplasmaeiweisse stattfindet und diese Denaturation von einer erhöhten Bindung der Hydroxylionen begleitet wird, so könnte die gesteigerte Acidität der geschädigten Zelle als direktes Resultat der Veränderung der sauer-alkalischen Eigenschaften der lebendigen Substanz selbst angesprochen werden. Damit wollen wir die Anwesenheit von Stoffwechsel-

verschiebungen, welche in dieser oder jener Weise die Ionenkonzentration in der beschädigten Zelle beeinflussen können, durchaus nicht leugnen; wir weisen nur auf die Veränderungen des Protoplasmaeiweisses hin und betrachten sie als eine der möglichen Quellen für die saure Reaktion. Dieser Umstand ist besonders wichtig, da RUNNSTRÖMS Untersuchungen (1935) gezeigt haben, dass die gebildete traumatische Säure nur mit grossen Schwierigkeiten auf irgendein bestimmtes intracellulöres Stoffwechselprodukt zurückzuführen ist; jedenfalls ist die bei der Beschädigung von *Arbacia*-Eiern sich entwickelnde Acidität, weder durch die Produktion von Milchsäure, noch durch die Proteolyse zu erklären.[1]

Nachdem wir nun zugegeben haben, dass bei der Beschädigung des Zellprotoplasmas ähnliche Veränderungen eintreten wie während der Eiweissdenaturation in vitro, können wir eine Reihe der sich hier entwickelnden Erscheinungen erklären oder näher erörtern. Bis zur letzten Zeit ist diese Deutung der verschiedenen reversiblen Zellbeschädigungen von den meisten Forschern bestritten worden (siehe z. B. HEILBRUNN 1928; WINTERSTEIN 1926; RUBINSTEIN 1934). Als Hauptargument diente die Irreversibilität der Eiweissdenaturation in vitro. Dieser Einspruch ist unseres Erachtens aus folgenden Gründen haltlos. Bekanntlich können einige denaturierende Agentien, wie z. B. Alkohol, Aceton, Herabsetzung der Salzkonzentration (beim Globulin) anfänglich eine vorübergehende Koagulation hervorrufen, die erst allmählich homodrom irreversibel wird. Die Untersuchungen der letzteren Zeit haben gezeigt, dass auch bei homodrom-irreversiblen Denaturationen eine Rückkehr des Eiweisses zu seinem nativen Ausgangszustande möglich ist. Zu diesem Zwecke hat man nicht nur den denaturierenden Faktor zu entfernen, sondern auch das gefällte Eiweiss mittels dieses oder jenen Stoffes zu behandeln. Als solche dienen gewöhnlich Basen, Säuren, Harnstoff und eine Reihe hydrotroper Salze Rhodanaten, Benzoaten, Salizylaten u. and. (MICHAELIS u. RONA 1910; WILLHEIM 1927, 1929; ANSON u. MIRSKY 1929, 1930, 1931; SPIEGEL-ADOLF 1929, 1932; PAULI u. WEISS 1931; BURK 1932 u. and.) Diese heterodrome Reversibilität ist nach folgenden Denaturationen mehr oder weniger vollständig erhalten worden: Hitze, Alkohol-, Aceton-, Säure-, Harnstoff-Denaturation, Denaturation durch Salze der Schwermetalle, durch Adsorbtion. Als heterodromirreversibel erwies sich die Strahlendenaturation. Es ist von Wichtigkeit, dass die Leichtigkeit, mit welcher das veränderte Eiweiss zu seinem Ausgangszustand heterodrom zurückkehrt, nicht nur von den Eigenschaften des denaturierenden Faktors, sondern auch von der Natur des Eiweisses selbst abhängt. Z. B. ist von den untersuchten Eiweissen das Serumalbumin am leichtesten reversibel. Am Hühneralbumin, welches bei den

[1] Es sei darauf hingewiesen, dass auch LEPESCHKIN (1937) die Acidose der absterbenden Zelle nicht dem gestörten Stoffwechsel, sondern dem Zerfall des Lipoproteinkomplex des Protoplasmas zuschreibt, welcher zum Freiwerden saurer Phosphatide führt.

VOL. 24 1943

gleichen Bedingungen denaturiert war, ist dieses Resultat fast unerreichbar. Eine Reihe von Eiweissen nimmt in dieser Hinsicht eine Mittelstellung zwischen den Serum- und Hühnereiweissalbuminen ein.

Wir vermuten, dass wir nicht das Recht haben bei reversiblen Zellbeschädigungen die Möglichkeit einer solchen heterodromen Wiederherstellung der lebendigen Substanz zu leugnen. Tatsächlich besteht die Beschädigungsreparation durchaus nicht in der Entfernung der alterierenden Agentien. Dieser Vorgang stellt eine aktive Auslösung der Lebenstätigkeit der Zelle dar, deren Energie auch eine heterodrome Rückkehr des alterierten Eiweisses zu seinem nativen Zustand hervorrufen kann. Obgleich wir den Zelleiweissen die Fähigkeit zuschreiben ähnliche Veränderungen zu erleiden wie die, welche im Eiweiss in vitro beobachtet werden, dürfen wir doch die Ausgangseigenschaften der lebendigen Eiweisse mit denen der widerstandsfähigen aus dem Organismus erhaltenen nicht identifizieren. Deshalb ist es sehr wahrscheinlich, dass die Neigung zur reversiblen Denaturation des Eiweisses der lebendigen Zelle stärker ausgedrückt ist als bei den bis jetzt untersuchten Proteinen.

Infolge des Vorhergesagten muss der Hinweis auf die Irreversibilität der Denaturation im Grunde für unrichtig und für das System der lebendigen Zelle als nicht anwendbar erklärt werden. Es steht uns somit nichts im wege die Möglichkeit einer Denaturation der Protoplasmaeiweisse sowohl bei irreversibler als auch bei reversibler Beschädigung anzuerkennen. Diese Folgerung wird uns ausserdem von einer Reihe von Tatsachen aufgezwungen, die im Laufe der Plasmaforschung der beschädigten Zellen registriert worden sind.

Die Feststellung der Denaturationsveränderungen bei der Beschädigung bedeutet noch nicht die Lösung der Frage, ob der alterierende Faktor stets die Rolle eines das Zelleiweiss unmittelbar denaturierenden Agenten spielt. Die Erörterung dieser Frage würde uns zu weit führen, da wir gezwungen wären den Wirkungsmechanismus jedes Faktors einzeln zu untersuchen. Infolgedessen beschränken wir uns auf einige allgemeine Betrachtungen.

Die Gegner der Denaturationslehre weisen gewöhnlich darauf hin, dass die Dosen, welche die Beschädigung der Zelle hervorrufen, für die Denaturation der Eiweisse in vitro nicht genügen. So hält es HEILBRUNN (1928) für unmöglich die beim Erwärmen erhaltene Protoplasmakoagulation durch die koagulierende Wirkung der hohen Temperatur auf die intracellulären Eiweisse zu erklären. HEILBRUNN erinnert an die Irreversibilität der termischen Eiweissgerinnung und stützt sich auf die Beobachtung, laut welcher die Protoplasmakoagulation durch bedeutend niedrigere Temperaturen erzielt wird als die Koagulation reiner Eiweisspräparate. Während die Koagulation der Eiweisslösung laut den Angaben dieses Forschers eine Temperatur von über 50° C. erfordert, genügen 30°—35° C. (und sogar weniger) um dasselbe Resultat an Eiern vieler Meeresorganismen zu erhalten. Dieselben Argumente führt

auch RUBINSTEIN an (1934) und leugnet die Bedeutung der Eiweissgerinnung während der termischen Protoplosmakoagulationsprozesse.

Tatsächlich ist die „denaturierende" Dose sehr oft bedeutend höher als die die Zelle reversibel oder sogar irreversibel beschädigende. Diese Nichtübereinstimmung braucht aber nicht davon abzuhängen, dass der Wirkungsmechanismus des Agenten auf das Substrat ein verschiedener ist. Es könnten auch andere Gründe dafür bestehen. Unsere Vorstellungen von den „Denaturationsdosen" beruhen vorwiegend auf Beobachtungen an unser cellulären Eiweissen (Blutserum, Hühnereiweisse u. s. w.). Es ist naheliegend, dass diese Proteine überhaupt stabiler und widerstandsfähiger sind als die labilen intracellulären Eiweisse, welche beim Stoffwechselprozess des lebendigen Systems in Mitleidenschaft gezogen werden.

Die Denaturationsfähigkeit der intracellulären Eiweisse ist sehr ungenügend erforscht und beschränkt sich fast ausschliesslich auf Beobachtungen an Muskel- und zumteil an Leberproteine. Ausserdem hat es der Biochemiker stets mit den stabileren Fraktionen zu tun, welche beim Gewinnen der Eiweisse aus den Geweben und während der weiteren Behandlungen nicht koagulieren. Die ausserordentlich interessanten Beobachtungen von KÜHNE (1864) lehren, dass die Muskeleiweisse, die mit Hilfe der gewöhnlichen biochemischen Methoden untersucht werden, eine richtige Vorstellung von der Sensibilität und Labilität der lebendigen Muskeleiweise nicht geben können. Im Gegensatz zu den modernen, die verschiedenen Manipulationen bei der Fraktionsfällung und der Dialyse übertreibenden biochemischen Methoden, war KÜHNES Muskeleiweissgewinnung beim Frosch mit minimalsten Störungen des vitalen Eiweisszustandes verbunden. Es gelang diesem Autor ein durchsichtiges siropähnliches hoch sensibles „Muskelplasma" zu erhalten. Dasselbe koagulierte mit Leichtigkeit bei Zusatz von Säuren, Basen(!), bei Herabsetzung oder Steigerung der Salzkonzentration, bei mechanischer Einwirkung (Rühren mit einem Glasstabe). Ausserdem behielt das Muskelplasma seine flüssige Konsistenz nur bei niedrigen Temperaturen bei; bei Zimmertemperatur verwandelte es sich schon nach einigen Stunden in eine Gallerte. Seine Reaktion verschob sich dabei in die saure Seite. Zweifelsohne konnte KÜHNE an der Hand seiner Extraktionsmethode einen Muskelsaft gewinnen, der einen sehr unbeständigen Eiweisskomponenten enthielt.

Später haben HALLIBURTON (1887), FÜRTH (1895) aus den Muskeln und POHL (1906) aus der Leber sehr unbeständig bei niedriger Temperatur koagulierende Eiweisse extrahiert. Dennoch stand das hier gebrauchte Verfahren dem KÜHNE'schen nach und konnte die Intaktheit des Eiweisses in geringerem Masse garantieren.

Diese Forschungen sind leider gründlich vergessen und KÜHNE's Versuche in ihrer anfänglichen Untersuchungsweise nicht mehr wiederholt worden. Aber viele der modernen Beobachtungen zwingen uns KÜHNE Recht zu geben.

VOL. 24 1943

So hat z. B. DEUTICKE (1930) gezeigt, dass die Löslichkeit des Eiweisses aus dem Muskelbrei vom Frosch oder Kaninchen bei 5—10° C. schon nach einer Stunde abnimt. Im Froschbrei bei 20° C. betrug der Löslichkeitsrückgang nach 1 Stunde 14,76 %, im Kaninchenbrei glich er bei 35° C. 47,95 %.

Infolge des Obenerwähnten scheint es uns nicht richtig zu sein, die unmittelbare denaturierende Wirkung dieses oder jenes Faktors auf das Protoplasma der lebendigen Zelle zu leugnen, weil an einigen nichtzellulären Eiweissen dasselbe Resultat bei Anwendung höherer Dosen erzielt wird. Im Speziellen müsste man sich eher verwundern, dass die für den Frosch und das Kaninchen optimale Temperatur die Muskeleiweisse im lebendigen Zustand nicht denaturiert, als mit HEILBRUNN u. RUBINSTEIN die denaturierende Wirkung hoher beschädigender Temperaturen zu bestreiten.

Tatsächlich haben mehrere Autoren (KÜHNE, FÜRTH 1895, 1919; POHL, DEUTIKE) darauf hingewiesen, dass die aus dem Organismus extrahierten Eiweisse im Reagenzglase bei Temperaturen, welche für das lebendige Tier optimal sind, mehr oder weniger schnell denaturiert werden. Zur Erklärung dieses Widerspruches sind mehrere Meinungen geäussert worden. FÜRTH nimmt z. B. an, dass im Glase in den extrahierten Zelleiweissen neue, nicht präformierte, in der lebendigen Zelle fehlende, hoch sensible Eiweisse entstehen (das lösliche Myogenfibrin der Warmblüter). POHL behauptet, dass die leicht denaturierbaren Eiweisse im Körper der Warmblüter im nativen Zustande infolge der Anwesenheit irgend welcher besonderer, die Denaturation verhindernden Stoffe erhalten bleiben. Auf Grund einer Reihe von Mutmassungen, von denen an anderer Stelle die Rede sein wird, nehmen wir an, dass die Temperatur, bei welcher sich das Leben abspielt, auch im Innern des Organismus eine spontan verlaufende Denaturation der intracellulären Eiweisse hervorruft. Um nun diese Vermutung mit der Nativität der Zelleiweisse in Einklang zu bringen, müssen wir neben der spontanen Denaturation den entgegengesetzten Vorgang zugeben, nämlich die Wiederherstellung des nativen Zustandes der Eiweisse.

Dieser Prozess ist nur möglich wenn in der Zelle ein ununterbrochener, auf eine bestimmte Mikrostruktur sich stützender Stoff- und Energiewechsel stattfindet.

Wenn wir uns diese Beziehung zwischen Stoffwechsel und Substrat in der normalen Zelle vergegenwärtigen wollen, wird es uns schwer fallen diesen Vorgang als ausschliesslich für die lebendige Substanz zu denken, dessen nativen Eiweisszustand er zu unterhalten hat. Wir vermuten, dass das andauernde Verbleiben im nativen Zustande des unbeständigen Zelleiweisses ebenso das Resultat des hier sich abspielenden Stoffwechsels ist wie der Stoffwechsel selbst die unumgängliche Folge der Existenz dieses spontan denaturierten und wieder hergestellten Substrates darstellt. Mit anderen Worten betrachten wir

den Stoffwechsel grundsätzlich als Ausdruck einer ununterbrochenen Rückkehr des spontan denaturierten Eiweisses in seinen nativen Zustand.

Sollte der native Zustand der Protoplasmaeiweisse tatsächlich vom Stoffwechsel der lebendigen Zelle unterhalten werden, so ist damit dem schädigenden Faktor die Möglichkeit gegeben die Zelleiweissdenaturation nicht nur unmittelbar zu bewirken, sondern sie auch sekundär, durch Störung des normalen Stoffwechselprozesses zu bedingen. Als Beispiel einer solchen sekundären Denaturationswirkung dient die Erstickung der Zelle. Der Ersatz der Luft durch Wasserstoff bei normalem Luftdruck kann an und für sich die Eiweisslösung unmittelbar nicht denaturieren. Wir haben uns aber an einem enormen Tatsachenmaterial überzeugen können, dass die Wasserstoffatmosphäre — gleich den anderen schädigenden Agentien — den typischen Komplex der paranekrotischen Veränderungen hervorruft, der seinerseits auf eine erfolgte Denaturation der Protoplasmaeiweisse hindeutet. Im gegebenen Falle ist dieses Resultat auf die Störung des normalen Stoffwechsels infolge der Asfiktion zurückzuführen. Was nun die Mehrzahl der anderen Agentien anbetrifft, so liegt kein Grund vor ihre primäre denaturierende Wirkung auf das ganze Protoplasma oder auf seine einzelnen Komponente zu leugnen. Die primäre Beschädigung des Eiweisssubstrats muss auf den Stoffwechselzustand wirken, der mit der Verstärkung der substantiellen Protoplasmaveränderungen verzerrt, verlangsamt und schliesslich ganz eingestellt wird. Es könnte angenommen werden, dass die Anfangsstadien der Plasmadenaturationsveränderungen, welche keine bedeutende Systemschädigungen bedingen, infolge der erhöhten chemischen Aktivität der aminen und carboxylen Eiweissgruppen für das Stadium der erhöhten Lebenstätigkeit verantwortlich sind, die so oft in den ersten Etappen der Beschädigungswirkung beobachtet werden.

VOL. 24 1943

LITERATUR.

Aisenberg, E. G. 1939. De l'action des nonélactrolytes dissouts sur la teneur en eau des cellules ovaires (Pectinaria hyperborea et Asterias rubens). Arch. Russes d'Anatom., d'Histol. et d'Embryol. *22*, 86.

Alexandrov, W. J. 1932. Über die Bedeutung der oxydo-reduktiven Bedingungen für die vitale Färbung, mit besonderer Berücksichtigung der Kernfärbung in lebendigen Zellen. Protoplasma, *17*, 162.

— 1934. Der Einfluss der Strahlenenergie auf die Permeabilität der Zellen und Gewebe. Annal. d. Roentgenol. et Radiol. *13*, 470.

— 1939. Über die „Permeabilität". „Permeabilitätsproblem" Konferenzschriften der Moskauer Physiologen-, Biochemiker- und Pharmakologengesellschaft 13—17 Mai 1936.

Anson, M. L. and Mirsky, A. E. 1929. Protein coagulation and its reversal. The preparation of completely coagulated hemoglobin. Journ. Gen. Physiol. *13*, 121.

Anson, M. L. and Mirsky, A. E. 1930. Protein coagulation and its reversal. (The preparation of insoluble globin, soluble globin and heme.) Journ. Gen. Physiol. *13*, 469.

— 1931 a. Protein coagulation and its reversal. The identity of normal hemoglobin with the hemoglobin prepared by the reversal of coagulation, as determined by solubility tests. Journ. Gen. Physiol. *14*, 597.

— 1931 b. Protein coagulation and its reversal. Journ. Gen. Physiol. *14*, 605.

— 1931 c. Protein coagulation and its reversal. Serum albumin. Journ. Gen. Physiol. *14*, 725.

— 1931 d. The reversibility of protein coagulation. Journ. of Physic. Chem. *35*, 185.

Astbury, W. T., Dickinson, S. and Bailey, K. 1935. The X-ray interpretation of denaturation and the structure of the seed globulins. Bioch. Journ. *29*, 2351.

Astbury, W. T. 1936. X-ray studies of protein structure. Nature *137*, 803.

— 1938. Röntgenstrahlen-Abenteuer unter den Eiweisskörpern. Koll.-Zeitschr. *83*, 130.

Astbury, W. T. and Lomax, R. 1935. An X-ray study of the hydratation and denaturation of Proteins. Journ, of the Chem. Soc. June. 846.

Ball, G. H. 1927. Studies on Paramecium. III. The effects of vital dyes on Paramecium caudatum. Biol. Bull. *52*, 68.

Bancroft, W. D. and Rutzler, J. E. 1931. The denaturation of albumin. Journ. of Physic. Chem. *35*. 144.

Barker, A. 1933. The optical rotatory power of heat-denatured egg albumin. Journ. of Biol. Chem. *103*, 1.

Battelli, F. und Stern, L. 1913. Einfluss der Anaesthetica auf die Oxydone. Bioch. Z. *52*, 226.

Bechhold, H. 1907. Kolloidstudien mit der Filtrationsmethode. Z. f. Physikal. Chemie. *60*, 257.

Becker, W. 1936. Vitale Cytoplasma und Kernfärbungen. Protoplasma. *26*, 439.

Bigwood, E. 1927 a. De la perméabilité de la gelée de gélatine au chlorure de calcium. La perméabilité sélective en l'absence de membrane. C. R. Soc. Biol. Paris. *96*, 131.

— 1927 b. De la perméabilité de la gelée de gélatine au chlorure de calcium. Caractère de la perméabilité sélective en l'absence de membrane. C. R. Soc. Biol. Paris. *96*, 136.

— 1927 c. De la perméabilité de la gelée de gélatine au chlorure de calcium. Une conséquence de la perméabilité sélective en l'absence de membrane. L'hydrolyse d'une solution d'un sel neutre. C. R. Soc. Biol. Paris. *96*, 199.

Booth, N. 1930. The denaturation of proteins. VI. The titration of the basic and acidic groups in eggalbumin. Bioch. Journ. *24*, 518. .

Bovie, W. T. 1913 a. A preliminary note on the coagulation of proteins by ultraviolet light. Science N. S. *37*, 24.

— 1913 b. The temperature coefficient of the coagulation caused by ultraviolet light. Science N. S. *37*, 373.

Braun, A. und Iwanoff, M. 1933. Vitalfärbung des quergestreiften Muskelgewebes unter verschiedenen experimentellen Bedingungen. Archives Russes d'Anat., d'Histol. et d'Embryol. *12*, 27.

Bridgman, P. W. 1914. The coagulation of albumen by pressure. Journ. of Biol. Chem. *19*, 511.

Bridgman, P. W. and Conant, J. B. 1929. Irreversible transformations of organic compounds under high pressures. Proc. Nat. Acad. of Sc. U. S. A. *15*, 680.

Burk, N. F. 1932. Osmotic pressure molecular weight, and stability of serum albumin. J. Biol. Chem. *98*, 353.

Chambers, L. A. and Flosdorf, E. W. 1936. The denaturation of proteins by sound waves of audible frequencies. J. Biol. Chem. *114*, 75.

CHICK, H. and MARTIN, C. J. 1911. On the „heat-coagulation" of proteins. Part II. The action of hot water upon egg-albumen and the influence of acid and salts upon reaction velocity. J. of Physiol. *43*, 1.

— 1913. Die Hitzekoagulation der Eiweisskörper. Kolloidchem. Beihefte. *5*, 49.

CLARK, J. H. 1922. The reaction of ultraviolet light on egg albumin in relation to the isoelectric point. Amer. Journ. Physiol. *61*, 72.

— 1925. Studies on radiated proteins. I. Coagulation of egg albumin by ultraviolet light and heat. Amer. Journ. of Physiol. *73*, 649.

— 1935. The denaturation of egg albumin by ultraviolet radiation. J. of Gen. Physiol. *19*, 199.

— 1936. The effect of radiation on proteins. Biol. effects of radiat. Edit by Duggar B. M. Mc Grow-Hill book comp. New York and London. *1*, 303.

DEUTICKE, H. J. 1930. Kolloidzustandsänderungen der Muskelproteine beim Absterben und bei der Ermüdung. Pflüg. Arch. *224*, 1.

EDSALL, J. T. 1930. Studies in the physical chemistry of muscle globulin. II. On some physicochemical properties of muscle globulin (Myosin). Biol. Chem. *89*, 289.

FAY, M. and HENDRIX, B. 1931. The effect of acid denaturation upon the combining power of fibrinogen. J. of Biol. Chem. *93*, 667.

FERNAU, A. und PAULI, W. 1915. Über die Einwirkung der durchdringenden Radiumstrahlung auf anorganische und Biokolloide I. Bioch. Zeitschr. *70*, 426.

— 1922. Über die Einwirkung der durchdringenden Radiumstrahlung auf anorganische und Biokolloide III. Koll. Z. *30*, 6.

FERNAU, A. und SPIEGEL-ADOLF, M. 1929. Physikalisch-chemische Untersuchungen bestrahlter Proteine. V Mitt. Die Veränderungen des Seralbumins bei Einwirkung durchdringender Radiumstrahlung und ihre Beziehungen zur Hitze- und Lichtgerinnung. Bioch. Zeitschr. *204*, 14.

FEULGEN, R. 1912. Das Verhalten der echten Nucleinsäure zu Farbstoffen. I Mitt. Hoppe-Seyl. Zeitschr. *80*, 73.

— 1913. Das Verhalten der echten Nucleinsäure zu Farbstoffen. II Mitt. Hoppe-Seyl. Zeitschr. *84*, 309.

FINN, D. B. 1932. Denaturation of proteins in muscle juice by freezing. Proceed. of Roy. Soc. Ser. B. *111*, 396.

FISCHER, A. 1935. Die Bindung von Heparin an Eiweiss. Bioch. Z. *278*, 133.

FISCHER, M. 1927. Kolloidchemie der Wasserbindung. Dresden und Leipzig.

FLOSDORF, E. W. and CHAMBERS, L. A. 1935. An immunological study of the effects of intense sound vibrations on egg albumin. Journ. of Immunol. *28*, 297.

FREY, E. 1922. Der Einfluss von Alkaloiden auf die Wärmeflockung von Eiweisslösungen und seine Beziehung zur Alkaloidwirkung überhaupt. Arch. f. exper. Path. u. Pharm. *95*, 36.

v. FÜRTH, O. 1895. Über die Eiweisskörper des Muskelplasmas. Arch, f. exp. Path. u. Pharm. *36*, 231.

— 1919. Die Kolloidchemie des Muskels und ihre Beziehungen zu den Problemen der Kontraktion und der Starre. Ergebn. d. Physiol. Jahrg. *17*, 363.

GROWTHER, J. A. and FAIRBROTHER, J. A. V. 1927. The action of X-rays on colloids. Philos. Mag. 7 Ser. *4*, 325.

HALLIBURTON, W. D. 1887. On muscle-plasma. The Journ. of Physiol. *8*, 133.

HARDY, W. 1899. On the structure of cell protoplasma. Journ. of Physiol. *24*, 158.

HARDY, W. B. and GARDINER, S. 1910. Proteins of blood plasma. Journ. of Physiol. *40*, 68.

HECHT, K. 1912. Studien über den Vorgang der Plasmolyse. Biol. d. Pfl. *11*, 137.

HEILBRUNN, L. V. 1928. The colloid chemistry of protoplasma. Protopl. Monogr. Berlin.
— 1930. The surface precipitation reaction of living cells. Proc. Amer. Phil. Soc. *19*, 295.
— 1932. The colloid chemistry of protoplasm. Scientia, Décembre 1.
HENDRIX, B. M. and WHARTON, P. S. 1934. The effect of variation of pH upon the process of heat denaturation of egg albumin. J. Biol. Chem. *105*, 633.
HENDRIX, B. M. and WILSON, V. 1928. A comparison of the titration curves of coagulated and uncoagulated egg albumin. J. Biol. Chem. *79*, 389.
HENRI, V. et MAYER, A. 1904. Action des radiations du radium sur les colloïdes. Compt. rend. Soc. Biol. *56*, 229.
HERZFELD, E. und KLINGER, R. 1917. Weitere Untersuchungen zur Chemie der Eiweisskörper. Bioch. Z. *78*, 349.
HOFFMANN, C. 1932. Strahlenhärte und biologische Wirkung. (Untersuchungen an Protozoen mittels Vitalfärbung.) Strahlenther. *43*, 140.
HOPKINS, F. G. 1930. Denaturation of proteins by urea and related substances. Nature. *126*, 328.
KAMNEV, I. 1934 a. The alteration of the epitheliel cells of animals under the effect of distilled water and Ringer salt solution. Trans. of the Physiol. Inst. of the University of Leningrad. *14*, 166.
— 1934 b. Der Einfluss von hypo- und hypertonischen Lösungen auf die Struktur und Vitalfärbung der Epithelzellen des Amphibiendarmes (Rana temporaria, Triton taeniatus). Protoplasma. *21*, 169.
— 1936. The influence of isotonic solutions of sugar on various epitheliums of amphibia. Transact. of the Physiol. Inst. of the University of Leningrad. *16*, 127.
— 1938. The permeability to sugars of striped muscles of frog. Arch. Russ. d'Anat., d'Histol. et d'Embr. *19*, 145.
KELLER, R. 1932. Die Elektrizität in der Zelle. Mährisch-Ostrau.
KIESEL, A. 1930. Chemie des Protoplasmas. Protoplasma-Monographie. Berlin.
KIYOTAKI, U. 1922. Nephelometrische Studien über den Einfluss der Temperaturerhöhung auf Serum und Plasma. Bioch. Z. *128*, 354.
KÜHNE, W. 1864. Untersuchungen über das Protoplasma und die Contractilität. Leipzig.
KÜSTER, E. 1926. Über vitale Protoplasmafärbung. Zeitschr. f. wiss. Mikr. *43*, 378.
LAPICQUE, L. 1925. La cellule, est-elle enveloppée d'une membrane semi-perméable? Ann. d. Physiol. et Physico-chimie biol. *1*, 85.
LEPESCHKIN, W. W. 1923. Über das Wesen der Koagulation der Eiweissstoffe durch Alkohol und andere organische Substanzen. Koll-Zeitschr. *32*, 100.
— 1936. Fortschritte der Kolloidchemie des Protoplasmas in den letzten zehn Jahren II. Protoplasma. *25*, 124.
— 1937. Zell-Nekrobiose und Protoplasma-Tod. Protoplasma Monograph. Berlin.
LEWIS, P. S. 1927. The heat-denaturation of proteins. Part IV. The free basic and acidic groups of fresh and denatured haemoglobin. Bioch. Journ. *21*, 46.
LIEBERMANN, L. 1891. Studien über die chemischen Processe in der Magenschleimhaut. Arch. f. d. ges. Physiol. *50*, 25.
LOUGHLIN, W. I. 1933. The denaturation of proteins. X. The acid and alkalititration curves of crystalline egg-albumin. Bioch. Journ. *27*, 99.
MAGAT, M. 1939. Die Permeabilität der normalen und Krebszellen. „Permeabilitätsproblem". Konferenzschriften der Moskauer Physiologen-, Biochemiker- und Pharmakologengesellschaft. 13—17 Mai 1936.
MAINZER, F. 1932. Untersuchungen an einem Bence-Jones-Protein. III Mit. Der isoelektrische Punkt des denaturierten Bence-Jones-Proteins. Bioch. Z. *246*, 156.

MAKAROV, P. 1934. De l'action des substances narcotiques sur les cellules épithéliales. Arch. Russes d'Anat., d'Histol. et d'Embryol. *13*, 199.

— 1935. Experimentelle Untersuchungen an Protozoen mit Bezug auf das Narkose-Problem. Protoplasma. *24*, 593.

— 1936. Die vitalen Veränderungen der Nervenzelle unter der Einwirkung von Narcotica. Arch. Russes d'Anat., d'Histol. et d'Embriol. *15*, 111.

— 1938. The problem of general and cellular narcosis. Arch. Russes d'Anat., d'Histol. et d'Embriol. *19*, 269.

MESTSCHERSKAJA, K. 1935. Über die Eigentümlichkeiten der Reaktion der sexuellen Zellen auf die Einwirkung von Agentien in subletalen Dosen. Arch. d. Sc. Biologiques, Ser. B. *37*, 836.

MICHAELIS, L. 1920. Der heutige Stand der allgemeinen Theorie der histologischen Färbung. Arch. f. mikr. Anat. *94*, 580.

MICHAELIS, L. und DAVIDSOHN, H. 1911. Der isoelektrische Punkt des genuinen und des denaturierten Serumalbumins. Bioch. Z. *33*, 456.

MICHAELIS, L. und RONA, P. 1909. Elektrochemische Alkalinitätsmessungen an Blut und Serum. Bioch. Zeitschr. *18*, 317.

— 1910. Beiträge zur allgemeinen Eiweisschemie. III Mitt. Die Denaturierung des Serumalbumins. Bioch. Z. *29*, 494.

V. MÖLLENDORFF, W. 1920. Vitale Färbung an tierischen Zellen. Grundlagen, Ergebnisse und Ziele biologische Farbstoffversuche. Ergebnisse d. Physiol. *18*, 141.

— 1921. Methoden zu Studien über vitale Färbungen an Tierzellen. Abderh. Handbuch. d. biol. Arbeitsmeth. Abt. V. *2*, 97.

MOND, R. 1922. Untersuchungen über die Wirkung der ultravioletten Strahlen auf Eiweisslösungen I Mitt. Pflüg. Arch. *196*, 540.

— 1923. Untersuchungen über die Wirkung der ultravioletten Strahlen auf Eiweisslösungen II Mitt. Pflüg. Arch. *200*, 374.

MOOR, B. and ROAF, H. 1913. Der osmotische Druck der Kolloide und seine biologische Bedeutung. Koll.-Zeitschr. *13*, 133.

NAKASHIMA, Y. 1927. Einige Versuche zum Grundvorgang der biologischen Strahlenwirkung. Strahlenther. *24*, 1.

NASSONOV, D. 1930. Über den Einfluss der Oxydationsprozesse auf die Verteilung von Vitalfarbstoffen in der Zelle. Zeitschr. f. Zellforsch. u. mikr. Anat. *11*, 179.

— 1932 a. Über die Ursachen der reversiblen Gelatinierung des Zellkernes. Protoplasma. *15*, 239.

— 1932 b. Vitalfärbung des Makronukleus aerober und anaerober Infusorien. Protoplasma. *17*, 218.

— 1938. The influence of nonelectrolytes on the water contents in live muscles and in swelling gelatin. Arch. Russes d'Anat., d'Histol. et d'Embryol. *19*, 298.

— 1939. Die Membrankonzeption in der Permeabilitätslehre. „Permeabilitätsproblem." Konferenzschriften der Moskauer Physiologen-, Biochemiker- und Pharmakologengesellschaft. 13—17 Mai 1936.

NASSONOV, D. et AISENBERG, E. 1937. L'influence des non électrolytes sur la teneur en eau des muscles vivants et tués. Annales de Physiol. *13*, 1179.

NASSONOV, D. and ALEXANDROV, W. 1934. Arch. d. scienc. Biolog. *36*, Ser. A, 95.

— 1938. Mécanisme de l'action toxique des substances sur le protoplasma. Annal. d. Physiol. *14*, 816.

— 1940. Die Reaktion der lebenden Substanz auf äussere Einwirkungen. (Die Denaturationstheorie der Beschädigung und Reizung.) Verlag d. Acad. d. Wissensch. U. S. S. R. Moskau-Leningrad.

VOL. 24 1943

PALTAUF, A. 1928. Die Lebendfärbung von Zellkornen. Sitzungsb. Akad. d. Wiss. in Wien. Abt. I. *137*, 691.

PARAT, M. 1928. Contribution à l'étude morphologique et physiologique du cytoplasma. Chondriome, vacuome (Appareil de Golgi), enclaves etc. ph, oxydases, peroxydases, rh de la cellule animale. Arch. d'Anat. microscop. *24*, F. II et III.

PAULI, W. und VALKO, E. 1933. Kolloidchemie der Eiweisskörper. Dresden und Leipzig.

PAULI, W. und WEISS, R. 1931. Über Beziehungen zwischen Kolloiden und konstitutiven Änderungen einiger Proteine I. Bioch. Z. *233*, 381.

PEDERSEN, K. O. 1931. Temperature stability and denaturation of serum albumin. Nature. *128*, 150.

POHL, J. 1906. Über Organeiweiss. Beitr. z. chem. Physiol. u. Pathol. *7*, 381.

PORAI-KOSCHITZ, A. E. 1937. Reserches on the theory of silk and wool dyeing. Bulletin d. l'Acad. d. Sc. de l'U. R. S. S. Classe d. sc. techniques.

PORAI-KOSCHITZ, A E., WELLER, E. A. und SOKOLOWA, N. W. 1937. Zur Theorie des Färbeprozesses. Arbeiten des L. K. Ch. T. I. *5*.

PORAI-KOSCHITZ, A. E., WELLER, E. A., SOKOLOWA, N. W. und DMITRIEWA, L. C. 1937. Zur Theorie des Färbeprozesses. Mitt. IX. Arbeiten des L. K. Ch. T. I. *5*.

PORCHER, CH. 1925. Les divers complexes caséinate de chaux + phosphate de chaux et leur façon de se comporter vis-à-vis de la présure. C. R. l'Acad. Sc. *180*, 1534.

PRIWOLNEV. T. J. 1935. Der Einfluss der hohen Temperatur auf verschiedene Entwicklungsstadien des Leiches des Flussbarsches. (Perca fluviatilis.) Travaux d. l'Instit. Biologique de Peterhof. *13/14*, 45.

QUAGLIARIELLO, G. 1912. Die Änderung der Wasserstoffionenkonzentration während der Hitzekoagulation der Proteine. Bioch. Z. *44*, 157.

RAJEWSKY, B. 1930. Die Wirkungen der kurzwelligen Strahlen auf Eiwesskörper. I Mitt. Bioch. Z. *227*, 272.

RAMSDEN, W. 1894. Die Coagulierung von Eiweisskörpern auf mechanischem Wege. Arch. f. Physiol. *517*.

— 1903. Separation of solids in the surface-layers of solutions and „suspensions". Proc. Roy. Soc. *72*, 156.

— 1904. Abscheidung fester Körper in den Oberflächenschichten von Lösungen und „Suspensionen". Zeitschr. f. Physial-Chemie. *47*, 336.

RIES, E. 1937. Untersuchungen über den Zelltod. I. Die Veränderungen der Färbbarkeit beim Absterben der Zellen, mit besonderer Berücksichtigung der vitalen und postvitalen Kernfärbung. Zeitschr. f. Zellf. und mikr. Anat. *26*, 507.

RUBINSTEIN, D. L. 1934. Die Physikochemie der Eiweisse als Grundlage der Protoplasmaphysikochemie. „Eiweissproblem." Schriften der Eiweisskonferenz in Moskau.

RUNNSTRÖM, J. 1935. Acid formation in frozen and thawed Arbacia punctulata eggs and its possible bearing on the problem of activation. Biol. Bull. *69*, 345.

SCARTH, G. 1926. The mechanism of accumulation of dyes by living cells. Plant Physiol. *1*, 215.

SCHARNKE, S. 1922. Untersuchungen über die ausflockende Wirkung von Alkaloiden auf Eiweissstoffe in saurer, neutraler und alkalischer Lösung. Inaug.-Diss. Marburg.

SCHMIDT, E. 1921. Experimentelle und histologische Untersuchungen über den Einfluss der Röntgenstrahlen auf die vitale Färbbarkeit der Gewebe. Strahlenth. *12*, 517.

SCHMITT, F. O., JOHNSON, C. H. and OLSON, A. B. 1928. Effects of high frequency sound waves on Protoplasm. Proc. Soc. exp. Biol. and Med. *25*, 728.

SCHULZ, FR. 1898. Über Oxydation von krystallisiertem Eiereiweiss mit Wasserstoffsuperoxyd. Hoppe-Seyl. Zeitschr. *29*, 86.

SMOILOVSKAJA, E. 1938. The supravital staining of surviving section of normal tissues in physiological and hypotonic surroundings. Archives Russes d'Anat., d'Histol, et d'Embr. *19*, 296.

SOLITERMANN, P. 1935. Über die Veränderung der Vitalfärbung von Embryonalgeweben in Explantaten bei Unterdrückung der energieliefernden Prozesse. Arch. f. experim. Zellf. *17*, 106.

SÖRENSEN, S. P. L. und JÜRGENSEN, E. 1911. Über die Hitzekoagulation der Proteine. I Mitt. Wird die Wasserstoffionenkonzentration der Lösung durch die Koagulation geändert? Bioch. Z. *31*, 397.

SPIEGEL-ADOLF, M. 1927. Physikalisch-chemische Untersuchungen bestrahlter Proteine. I Mitt. Die Veränderungen des Seralbumins bei Ultraviolettbestrahlung und ihre Beziehungen zur Hitzegerinnung. Bioch. Z. *186*, 181.

— 1928. Neuere Ergebnisse über Eiweissveränderungen durch ultraviolette, Radium- und Röntgenstrahlen. Erg. d. Physiol. *27*, 832.

— 1932. Reversibility of protein denaturation in adsorption and elution. J. biol. Chem. *97*, 64.

— 1934. Effect of ultra-violet radiation and heat upon protein solutions of low concentrations. Bioch. Journ. *28*, 372.

STEARN, A. E. 1930 a. The nature of isoelectric gelatin in solution. Evidence for the existence of the ampholyte ion. I. Ionic displacement reactions. Journ. Physic. Chem. *34*, 972.

— 1930 b. The nature of isoelectric gelatin in solution. II. Conductivity titrations of gelatin with crystalviolet. Journ. Physic. Chem. *34*, 981.

STEARN, A. E. and STEARN, E. W. 1929. The mechanism of staining explained on a chemical basis. I. The reaction between dyes, proteins and nucleic acid. Stain Technol. *4*, 111.

STILES, W. 1937. The constitution of plant cell membranes. Transact, of the Faraday Soc. *33*, 923.

SULUTOV, L. 1937. Veränderungen im Zytoplasma und im Kern unter der Wirkung von Schwefelwasserstoff. Arch. Russes d'Anat., d'Histol. et d'Embryol. *17*, 103.

TRIFONOVA, A. 1935. Sur la physiologie de la différentiation et de la croissance. Arch. des Sc. Biolog. *37*, 757.

TROSCHIN, A. S. 1939. Über den Mechanismus der Schutzwirkung von Nichtelektrolyten bei Schädigung der Muskel durch Hypotonie. Arch. Russes d'Anat., d'Histologie et d'Embryol. *22*, I.

UMETSU, K. 1923. Untersuchungen über die Bindungsfähigkeit der Eiweisskörper für Farbstoffe. Bioch. Z. *137*, 258.

UTKINA, O. T. 1941. In Druck.

VALKO, E. 1937. Kolloidchemische Grundlagen der Textilveredlung. Berlin.

VICKERY, I. R. 1926. Studies on the refrigeration of meat. II. The freezing of beef and mutton press juices. Australian Journ. of exp. Biol. and Med. Sc. *3*, 81.

WALLBACH, G. 1931. Die Stellung der vitalen Diffusfärbung und der vitalen Kernfärbung unter den funktionellen Erscheinungen an der Zelle. Zeitschr. f. Zellf. u. mikr. Anat. *13*, 180.

WANKELL, FR. 1921. Über Reduktion basischer Farbstoffe im lebenden Protoplasma. Ber. d. naturforsch. Ges. Freiburg i. Br. *23*, 118.

WARBURG, O. 1926. Über die Wirkung von Blausäureäthylester (Äthylcarbylamin) auf die Pasteursche Reaktion. Bioch. Z. *172*, 432.

WELS, P. 1923. Der Einfluss der Röntgenstrahlen auf Eiweisskörper. Pflüg. Arch. *199*, 226.

VOL. 24 1943

WELS, P. 1924. Beiträge zur Kenntnis der Strahlenwirkungen. Strahlenther. *16*, 617.
— 1937. Über einige kolloidchemische und chemische Strahlenwirkungen und ihre biologische Bedeutung. Strahlenther. *60*, 355.
WELS, P. und THIELE, A. 1925. Der Einfluss der Röntgenstrahlen auf Eiweisskörper. II Mitt. Pflüg. Arch. *209*, 49.
WIECHOWSKI, W. 1917. Über „mechanische Denaturierung" von Eiweisskörpern und das Trocknen von Organen zwecks biologischer Untersuchung. Bioch. *81*. 278.
WILLHEIM, R. 1927. Zur Frage der Eiweisskörper mit Bence-Jonesscher Reaktion. Zugleich ein Beitrag zum Problem der Reversibilität der Hitzegerinnung. Bioch. Z. *180*, 231.
— 1929. Über eine, durch neutrale Salze bewirkte mit neuerlich Koagulationsfähigkeit einhergehende Lösung von Eiweisskoagulation. Koll.-Zeitschr. *68*, 217.
WINTERSTEIN, H. 1926. Die Narkose. Berlin.
WU, H. 1927. Studies on denaturation of proteins. II. Coagulation by alcohol. Chin. J. Physiol. *1*, 81.
WU, H. and CHEN, T. T. 1929. Studies on denaturation of proteins. VIII. Effect of denaturation and coagulation on acid- and base-binding power of proteins. Chin. J. Physiol. *3*, 7.
WU, H. and LIU, S. C. 1931. Coagulation of egg albumin by supersonic waves. Proc. Soc. exp. Biol. a. Med. *28*, 782.
WU, H. and YANG, E. F. 1931. Studies on denaturation of proteins XI. Effect of hydrogen ion concentration on rate of denaturation of egg albumin by urea. Chin. J. Physiol. *5*, 301.
YOUNG, E. G. 1922. The coagulation of protein by sunlight. Proc. of Roy. Soc. Ser. B. *93*, 235.

VOL
24
194

VOL.
24
1943